

**OLIGONUCLEOTIDE COMPOSITIONS AND METHODS FOR
THE MODULATION OF THE EXPRESSION OF B7 PROTEIN**

INTRODUCTION

This is a continuation-in-part of International Patent
5 Application No. PCT/US00/14471, which is a continuation-in-
part of U.S. Application Serial No. 09/326,186, filed June 4,
1999, which is a continuation-in-part of U.S. Application
Serial No. 08/777,266, filed December 31, 1996.

FIELD OF THE INVENTION

10 This invention relates to diagnostics, research reagents
and therapeutics for disease states which respond to
modulation of T cell activation. In particular, this
invention relates to antisense oligonucleotide interactions
with certain messenger ribonucleic acids (mRNAs) or DNAs
15 involved in the synthesis of proteins that modulate T cell
activation. Antisense oligonucleotides designed to hybridize
to nucleic acids encoding B7 proteins are provided. These
oligonucleotides have been found to lead to the modulation of
the activity of the RNA or DNA, and thus to the modulation of
20 T cell activation. Palliative, therapeutic and prophylactic
effects result.

BACKGROUND OF THE INVENTION

Inflammation is a localized protective response mounted
25 by tissues in response to injury, infection, or tissue
destruction resulting in the destruction of the infectious or
injurious agent and isolation of the injured tissue. A
typical inflammatory response proceeds as follows:
recognition of an antigen as foreign or recognition of tissue

damage, synthesis and release of soluble inflammatory mediators, recruitment of inflammatory cells to the site of infection or tissue damage, destruction and removal of the invading organism or damaged tissue, and deactivation of the system once the invading organism or damage has been resolved. In many human diseases with an inflammatory component, the normal, homeostatic mechanisms which attenuate the inflammatory responses are defective, resulting in damage and destruction of normal tissue.

Cell-cell interactions are involved in the activation of the immune response at each of the stages described above. One of the earliest detectable events in a normal inflammatory response is adhesion of leukocytes to the vascular endothelium, followed by migration of leukocytes out of the vasculature to the site of infection or injury. In general, the first inflammatory cells to appear at the site of inflammation are neutrophils, followed by monocytes and lymphocytes. Cell-cell interactions are also critical for activation of both B-lymphocytes (B cells) and T-lymphocytes (T cells) with resulting enhanced humoral and cellular immune responses, respectively.

The hallmark of the immune system is its ability to distinguish between self (host) and nonself (foreign invaders). This remarkable specificity exhibited by the immune system is mediated primarily by T cells. T cells participate in the host's defense against infection but also mediate organ damage of transplanted tissues and contribute to cell attack in graft-versus-host disease (GVHD) and some autoimmune diseases. In order to induce an antigen-specific immune response, a T cell must receive signals delivered by an antigen-presenting cell (APC). T cell-APC interactions can be divided into three stages: cellular adhesion, T cell receptor (TCR) recognition, and costimulation. At least two discrete signals are required from an APC for induction of T cell activation. The first signal is antigen-specific and is

a) b)

a) b)

methods for inhibiting T cell responses using a first agent which inhibits a costimulatory agent, such as an CTLA4-Ig fusion protein, and a second agent which inhibits cellular adhesion, such as an anti-LFA-1 antibody. Such methods are
5 indicated to be particularly useful for inhibiting the rejection of transplanted tissues or organs.

International Publication No. WO95/32734 discloses Fc RII bridging agents which either prevent the upregulation of B7 molecules or impair the expression of ICAM-3 on antigen
10 presenting cells. Such Fc γ RII bridging agents include proteins such as aggregated human IgG molecules or aggregated Fc fragments of human IgG molecules.

International Publication No. WO96/11279 discloses recombinant viruses comprising genetic sequences encoding (1)
15 one or more immunostimulatory agents, including B7-1 and B7-2, and (2) and antigens from a disease causing agent. Also disclosed are methods of treating diseases using such recombinant viruses.

To date, there are no known therapeutic agents which
20 effectively regulate and prevent the expression of B7 proteins such as B7-1 and B7-2. Thus, there is a long-felt need for compounds and methods which effectively modulate critical costimulatory molecules such as the B7 proteins.

25 SUMMARY OF THE INVENTION

In accordance with the present invention, oligonucleotides are provided which specifically hybridize with nucleic acids encoding B7-1 or B7-2. Certain oligonucleotides of the invention are designed to bind either
30 directly to mRNA transcribed from, or to a selected DNA portion of, the B7-1 or B7-2 gene, thereby modulating the amount of protein translated from a B7-1 or B7-2 mRNA or the amount of mRNA transcribed from a B7-1 or B7-2 gene, respectively.

Oligonucleotides may comprise nucleotide sequences sufficient in identity and number to effect specific hybridization with a particular nucleic acid. Such oligonucleotides are commonly described as "antisense."

5 Antisense oligonucleotides are commonly used as research reagents, diagnostic aids, and therapeutic agents.

It has been discovered that the *B7-1* and *B7-2* genes, encoding *B7-1* and *B7-2* proteins, respectively, are particularly amenable to this approach. As a consequence of
10 the association between *B7* expression and T cell activation and proliferation, inhibition of the expression of *B7-1* or *B7-2* leads to inhibition of the synthesis of *B7-1* or *B7-2*, respectively, and thereby inhibition of T cell activation and proliferation. Additionally, the oligonucleotides of the
15 invention may be used to inhibit the expression of one of several alternatively spliced mRNAs of a *B7* transcript, resulting in the enhanced expression of other alternatively spliced *B7* mRNAs. Such modulation is desirable for treating various inflammatory or autoimmune disorders or diseases, or
20 disorders or diseases with an inflammatory component such as asthma, juvenile diabetes mellitus, myasthenia gravis, Graves' disease, rheumatoid arthritis, allograft rejection, inflammatory bowel disease, multiple sclerosis, psoriasis, lupus erythematosus, systemic lupus erythematosus, diabetes,
25 multiple sclerosis, contact dermatitis, rhinitis, various allergies, and cancers and their metastases. Such modulation is further desirable for preventing or modulating the development of such diseases or disorders in an animal suspected of being, or known to be, prone to such diseases or
30 disorders. The invention also relates to pharmaceutical compositions which comprise an antisense oligonucleotide to a *B7* protein in combination with a second anti-inflammatory agent, such as a second antisense oligonucleotide to a protein which mediates intercellular interactions, e.g., an
35 intercellular adhesion molecule (ICAM) protein.

Methods comprising contacting animals with oligonucleotides specifically hybridizable with nucleic acids encoding B7 proteins are herein provided. These methods are useful as tools, for example, in the detection and
5 determination of the role of B7 protein expression in various cell functions and physiological processes and conditions, and for the diagnosis of conditions associated with such expression. Such methods can be used to detect the expression of B7 genes (i.e., B7-1 or B7-2) and are thus believed to be
10 useful both therapeutically and diagnostically. Methods of modulating the expression of B7 proteins comprising contacting animals with oligonucleotides specifically hybridizable with a B7 gene are herein provided. These methods are believed to be useful both therapeutically and diagnostically as a
15 consequence of the association between B7 expression and T cell activation and proliferation. The present invention also comprises methods of inhibiting B7-associated activation of T cells using the oligonucleotides of the invention. Methods of treating conditions in which abnormal or excessive T cell
20 activation and proliferation occurs are also provided. These methods employ the oligonucleotides of the invention and are believed to be useful both therapeutically and as clinical research and diagnostic tools. The oligonucleotides of the present invention may also be used for research purposes.
25 Thus, the specific hybridization exhibited by the oligonucleotides of the present invention may be used for assays, purifications, cellular product preparations and in other methodologies which may be appreciated by persons of ordinary skill in the art.

30 The methods disclosed herein are also useful, for example, as clinical research tools in the detection and determination of the role of B7-1 or B7-2 expression in various immune system functions and physiological processes and conditions, and for the diagnosis of conditions associated
35 with their expression. The specific hybridization exhibited

by the oligonucleotides of the present invention may be used for assays, purifications, cellular product preparations and in other methodologies which may be appreciated by persons of ordinary skill in the art. For example, because the
5 oligonucleotides of this invention specifically hybridize to nucleic acids encoding B7 proteins, sandwich and other assays can easily be constructed to exploit this fact. Detection of specific hybridization of an oligonucleotide of the invention with a nucleic acid encoding a B7 protein present in a sample
10 can routinely be accomplished. Such detection may include detectably labeling an oligonucleotide of the invention by enzyme conjugation, radiolabeling or any other suitable detection system. A number of assays may be formulated employing the present invention, which assays will commonly
15 comprise contacting a tissue or cell sample with a detectably labeled oligonucleotide of the present invention under conditions selected to permit hybridization and measuring such hybridization by detection of the label, as is appreciated by those of ordinary skill in the art.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph showing the inhibitory effect of the indicated oligonucleotides on B7-1 protein expression in COS-7 cells.

25 Figure 2 is a dose-response curve showing the inhibitory effect of oligonucleotides on cell surface expression of B7-1 protein. Solid line, ISIS 13812; dashed line, ISIS 13800; dotted line, ISIS 13805.

Figure 3 is a bar graph showing the inhibitory effect
30 of the indicated oligonucleotides on cell surface expression of B7-2 in COS-7 cells.

Figure 4 is a bar graph showing the inhibitory effect of the indicated oligonucleotides, including ISIS 10373 (a 20-mer) and ISIS 10996 (a 15-mer) on cell surface expression of
35 B7-2 in COS-7 cells.

Figure 5 is a bar graph showing the specificity of inhibition of B7-1 or B7-2 protein expression by oligonucleotides. Cross-hatched bars, B7-1 levels; striped bars, B7-2 levels.

5 Figure 6 is a dose-response curve showing the inhibitory effect of oligonucleotides having antisense sequences to ICAM-1 (ISIS 2302) or B7-2 (ISIS 10373) on cell surface expression of the ICAM-1 and B7-2 proteins. Solid line with X's, levels of B7-1 protein on cells treated with ISIS 10373; dashed line
10 with asterisks, levels of ICAM-1 protein on cells treated with ISIS 10373; solid line with triangles, levels of B7-1 protein on cells treated with ISIS 2302; solid line with squares, levels of ICAM-1 protein on cells treated with ISIS 10373.

Figure 7 is a bar graph showing the effect of the
15 indicated oligonucleotides on T cell proliferation.

Figure 8 is a dose-response curve showing the inhibitory effect of oligonucleotides on murine B7-2 protein expression in COS-7 cells. Solid line with asterisks, ISIS 11696; dashed line with triangles, ISIS 11866.

20 Figure 9 is a bar graph showing the effect of oligonucleotides ISIS 11696 and ISIS 11866 on cell surface expression of murine B7-2 protein in IC-21 cells. Left (black) bars, no oligonucleotide; middle bars, 3 μ M indicated oligonucleotide; right bars, 10 μ M indicated oligonucleotide.

25 Figure 10 is a graph showing the effect of ISIS 17456 on severity of EAE at various doses.

DETAILED DESCRIPTION OF THE INVENTION

The present invention employs oligonucleotides for use
30 in antisense inhibition of the function of RNA and DNA encoding B7 proteins including B7-1 and B7-2. The present invention also employs oligonucleotides which are designed to be specifically hybridizable to DNA or messenger RNA (mRNA) encoding such proteins and ultimately to modulate the amount

of such proteins transcribed from their respective genes. Such hybridization with mRNA interferes with the normal role of mRNA and causes a modulation of its function in cells. The functions of mRNA to be interfered with include all vital
5 functions such as translocation of the RNA to the site for protein translation, actual translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of such
10 interference with mRNA function is modulation of the expression of a B7 protein, wherein "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a B7 protein. In the context of the present invention, inhibition is the preferred form of modulation of
15 gene expression.

Oligonucleotides may comprise nucleotide sequences sufficient in identity and number to effect specific hybridization with a particular nucleic acid. Such oligonucleotides which specifically hybridize to a portion of
20 the sense strand of a gene are commonly described as "antisense." Antisense oligonucleotides are commonly used as research reagents, diagnostic aids, and therapeutic agents. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often
25 used by those of ordinary skill to elucidate the function of particular genes, for example to distinguish between the functions of various members of a biological pathway. This specific inhibitory effect has, therefore, been harnessed by those skilled in the art for research uses.

30 The specificity and sensitivity of oligonucleotides is also harnessed by those of skill in the art for therapeutic uses. For example, the following U.S. patents demonstrate palliative, therapeutic and other methods utilizing antisense oligonucleotides. U. S. Patent 5,135,917 provides antisense
35 oligonucleotides that inhibit human interleukin-1 receptor

expression. U.S. Patent 5,098,890 is directed to antisense oligonucleotides complementary to the *c-myb* oncogene and antisense oligonucleotide therapies for certain cancerous conditions. U.S. Patent 5,087,617 provides methods for
5 treating cancer patients with antisense oligonucleotides. U.S. Patent 5,166,195 provides oligonucleotide inhibitors of HIV. U.S. Patent 5,004,810 provides oligomers capable of hybridizing to herpes simplex virus Vmw65 mRNA and inhibiting replication. U.S. Patent 5,194,428 provides antisense
10 oligonucleotides having antiviral activity against influenza virus. U.S. Patent 4,806,463 provides antisense oligonucleotides and methods using them to inhibit HTLV-III replication. U.S. Patent 5,286,717 provides oligonucleotides having a complementary base sequence to a portion of an
15 oncogene. U.S. Patent 5,276,019 and U.S. Patent 5,264,423 are directed to phosphorothioate oligonucleotide analogs used to prevent replication of foreign nucleic acids in cells. U.S. Patent 4,689,320 is directed to antisense oligonucleotides as antiviral agents specific to CMV. U.S. Patent 5,098,890
20 provides oligonucleotides complementary to at least a portion of the mRNA transcript of the human *c-myb* gene. U.S. Patent 5,242,906 provides antisense oligonucleotides useful in the treatment of latent EBV infections.

Oligonucleotides capable of modulating the expression
25 of B7 proteins represent a novel therapeutic class of anti-inflammatory agents with activity towards a variety of inflammatory or autoimmune diseases, or disorders or diseases with an inflammatory component such as asthma, juvenile diabetes mellitus, myasthenia gravis, Graves' disease,
30 rheumatoid arthritis, allograft rejection, inflammatory bowel disease, multiple sclerosis, psoriasis, lupus erythematosus, systemic lupus erythematosus, diabetes, multiple sclerosis, contact dermatitis, eczema, atopic dermatitis, seborrheic dermatitis, nummular dermatitis, generalized exfoliative
35 dermatitis, rhinitis and various allergies. In addition,

oligonucleotides capable of modulating the expression of B7 proteins provide a novel means of manipulating the *ex vivo* proliferation of T cells.

It is preferred to target specific genes for antisense
5 attack. "Targeting" an oligonucleotide to the associated nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed
10 from the gene) whose expression is associated with a particular disorder or disease state, or a foreign nucleic acid from an infectious agent. In the present invention, the target is a cellular gene associated with several immune system disorders and diseases (such as inflammation and
15 autoimmune diseases), as well as with ostensibly "normal" immune reactions (such as a host animal's rejection of transplanted tissue), for which modulation is desired in certain instances. The targeting process also includes determination of a region (or regions) within this gene for
20 the oligonucleotide interaction to occur such that the desired effect, either detection or modulation of expression of the protein, will result. Once the target region have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well
25 and with sufficient specificity to give the desired effect.

Generally, there are five regions of a gene that may be targeted for antisense modulation: the 5' untranslated region (hereinafter, the "5'-UTR"), the translation initiation codon region (hereinafter, the "tIR"), the open reading frame
30 (hereinafter, the "ORF"), the translation termination codon region (hereinafter, the "tTR") and the 3' untranslated region (hereinafter, the "3'-UTR"). As is known in the art, these regions are arranged in a typical messenger RNA molecule in the following order (left to right, 5' to 3'): 5'-UTR, tIR,
35 ORF, tTR, 3'-UTR. As is known in the art, although some

Figure 1. The structure of the proposed model.

(in eukaryotes) or formylmethionine (prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions, in order to generate related polypeptides having different amino terminal sequences (Markussen et al., *Development*, 1995, 121, 3723; Gao et al., *Cancer Res.*, 1995, 55, 743; McDermott et al., *Gene*, 1992, 117, 193; Perri et al., *J. Biol. Chem.*, 1991, 266, 12536; French et al., *J. Virol.*, 1989, 63, 3270; Pushpa-Rekha et al., *J. Biol. Chem.*, 1995, 270, 26993; Monaco et al., *J. Biol. Chem.*, 1994, 269, 347; DeVirgilio et al., *Yeast*, 1992, 8, 1043; Kanagasundaram et al., *Biochim. Biophys. Acta*, 1992, 1171, 198; Olsen et al., *Mol. Endocrinol.*, 1991, 5, 1246; Saul et al., *Appl. Environ. Microbiol.*, 1990, 56, 3117; Yaoita et al., *Proc. Natl. Acad. Sci. USA*, 1990, 87, 7090; Rogers et al., *EMBO J.*, 1990, 9, 2273). In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA molecule transcribed from a gene encoding a B7 protein, regardless of the sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a

translation termination codon.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes
5 oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because
10 of desirable properties such as, for example, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nucleases.

Specific examples of some preferred modified oligonucleotides envisioned for this invention include those
15 containing phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioates and those with $\text{CH}_2\text{-NH-O-CH}_2$, $\text{CH}_2\text{-N(CH}_3\text{)-O-CH}_2$
20 [known as a methylene(methylimino) or MMI backbone], $\text{CH}_2\text{-O-N(CH}_3\text{)-CH}_2$, $\text{CH}_2\text{-N(CH}_3\text{)-N(CH}_3\text{)-CH}_2$ and $\text{O-N(CH}_3\text{)-CH}_2\text{-CH}_2$ backbones, wherein the native phosphodiester backbone is represented as O-P-O-CH_2). Also preferred are oligonucleotides having morpholino backbone structures (U.S. Patent 5,034,506).
25 Further preferred are oligonucleotides with $\text{NR-C(*)-CH}_2\text{-CH}_2$, $\text{CH}_2\text{-NR-C(*)-CH}_2$, $\text{CH}_2\text{-CH}_2\text{-NR-C(*)}$, $\text{C(*)-NR-CH}_2\text{-CH}_2$ and $\text{CH}_2\text{-C(*)-NR-CH}_2$ backbones, wherein "*" represents O or S (known as amide backbones; PCT WO92/20823). In other preferred embodiments, such as the peptide nucleic acid (PNA) backbone,
30 the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleobases being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen et al., *Science*, 1991, 254, 1497; U.S. Patent No. 5,539,082). Other preferred modified
35 oligonucleotides may contain one or more substituted sugar

moieties comprising one of the following at the 2' position:
OH, SH, SCH₃, F, OCN, OCH₃OCH₃, OCH₃O(CH₂)_nCH₃, O(CH₂)_nNH₂ or
O(CH₂)_nCH₃ where n is from 1 to about 10; C₁ to C₁₀ lower alkyl,
alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl;
5 Br; CN; CF₃; OCF₃; O-, S-, or N-alkyl; O-, S-, or N-alkenyl;
SOCH₃; SO CH₂; ₃ONO; ₂NO; N₂; NH; heterocycloalkyl;
heterocycloalkaryl; aminoalkylamino; polyalkylamino;
substituted silyl; an RNA cleaving group; a reporter group;
an intercalator; a group for improving the pharmacokinetic
10 properties of an oligonucleotide; or a group for improving the
pharmacodynamic properties of an oligonucleotide and other
substituents having similar properties. A preferred
modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also
known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv.*
15 *Chim. Acta*, 1995, 78, 486-504) i.e., an alkoxyalkoxy group.
A further preferred modification includes
2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also
known as 2'-DMAOE, as described in examples hereinbelow, and
2'-dimethylamino-ethoxyethoxy (also known in the art as
20 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e.,
2'-O-CH₂-O-CH₂-N(CH₂)₂, also described in examples hereinbelow.
(Martin et al., *Helv. Chim. Acta*, 1995, 78, 486). Other
preferred modifications include 2'-methoxy (2'-O-CH₃), 2'-
propoxy (2'-OCH₂CH₂CH₃) and 2'-fluoro (2'-F). Similar
25 modifications may also be made at other positions on the
oligonucleotide, particularly the 3' position of the sugar on
the 3' terminal nucleotide and the 5' position of the 5'
terminal nucleotide. Oligonucleotides may also have sugar
mimetics such as cyclobutyls in place of the pentofuranosyl
30 group.

The oligonucleotides of the invention may additionally
or alternatively include nucleobase modifications or
substitutions. As used herein, "unmodified" or "natural"
nucleobases include adenine (A), guanine (G), thymine (T),
35 cytosine (C) and uracil (U). Modified nucleobases include

nucleobases found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-methylcytosine, 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentiobiosyl HMC, as well synthetic nucleobases, e.g., 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N⁶(6-aminohexyl)adenine and 2,6-diaminopurine (Kornberg, A., DNA Replication, 1974, W.H. Freeman & Co., San Francisco, 1974, pp. 75-77; Gebeyehu, G., et al., *Nucleic Acids Res.*, 1987, 15, 4513).

Another preferred additional or alternative modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more lipophilic moieties which enhance the cellular uptake of the oligonucleotide. Such lipophilic moieties may be linked to an oligonucleotide at several different positions on the oligonucleotide. Some preferred positions include the 3' position of the sugar of the 3' terminal nucleotide, the 5' position of the sugar of the 5' terminal nucleotide, and the 2' position of the sugar of any nucleotide. The N⁶ position of a purine nucleobase may also be utilized to link a lipophilic moiety to an oligonucleotide of the invention (Gebeyehu, G., et al., *Nucleic Acids Res.*, 1987, 15, 4513). Such lipophilic moieties include but are not limited to a cholesteryl moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Lett.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-

phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantane
5 acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923). Oligonucleotides comprising
10 lipophilic moieties, and methods for preparing such oligonucleotides, as disclosed in U.S. Patents No. 5,138,045, No. 5,218,105 and No. 5,459,255, the contents of which are hereby incorporated by reference.

The present invention also includes oligonucleotides
15 which are chimeric oligonucleotides. "Chimeric" oligonucleotides or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least
20 one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a
25 substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency
30 of antisense inhibition of gene expression. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. By way of example, such "chimeras" may be "gapmers," i.e., oligonucleotides in which

5 a central portion (the "gap") of the oligonucleotide serves as a substrate for, e.g., RNase H, and the 5' and 3' portions (the "wings") are modified in such a fashion so as to have greater affinity for the target RNA molecule but are unable to support nuclease activity (e.g., 2'-fluoro- or 2'-methoxyethoxy substituted). Other chimeras include "wingmers," that is, oligonucleotides in which the 5' portion of the oligonucleotide serves as a substrate for, e.g., RNase H, whereas the 3' portion is modified in such a fashion so as to have greater affinity for the target RNA molecule but is unable to support nuclease activity (e.g., 2'-fluoro- or 2'-methoxyethoxy substituted), or vice-versa.

15 The oligonucleotides in accordance with this invention preferably comprise from about 8 to about 30 nucleotides. It is more preferred that such oligonucleotides comprise from about 15 to 25 nucleotides. As is known in the art, a nucleotide is a base-sugar combination suitably bound to an adjacent nucleotide through a phosphodiester, phosphorothioate or other covalent linkage.

20 The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives.

30 The oligonucleotides of the present invention can be utilized as therapeutic compounds, diagnostic tools and as research reagents and kits. The term "therapeutic uses" is intended to encompass prophylactic, palliative and curative uses wherein the oligonucleotides of the invention are contacted with animal cells either *in vivo* or *ex vivo*. When 35 contacted with animal cells *ex vivo*, a therapeutic use

includes incorporating such cells into an animal after treatment with one or more oligonucleotides of the invention. While not intending to be bound to a particular utility, the ex vivo modulation of, e.g., T cell proliferation by the
5 oligonucleotides of the invention can be employed in, for example, potential therapeutic modalities wherein it is desired to modulate the expression of a B7 protein in APCs. As an example, oligonucleotides that inhibit the expression of B7-1 proteins are expected to enhance the availability of
10 B7-2 proteins on the surface of APCs, thus increasing the costimulatory effect of B7-2 on T cells ex vivo (Levine et al., *Science*, 1996, 272, 1939).

For therapeutic uses, an animal suspected of having a disease or disorder which can be treated or prevented by
15 modulating the expression or activity of a B7 protein is, for example, treated by administering oligonucleotides in accordance with this invention. The oligonucleotides of the invention can be utilized in pharmaceutical compositions by adding an effective amount of an oligonucleotide to a suitable
20 pharmaceutically acceptable diluent or carrier. Workers in the field have identified antisense, triplex and other oligonucleotide compositions which are capable of modulating expression of genes implicated in viral, fungal and metabolic diseases. Antisense oligonucleotides have been safely
25 administered to humans and several clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic instrumentalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

30 The oligonucleotides of the present invention can be further used to detect the presence of B7-specific nucleic acids in a cell or tissue sample. For example, radiolabeled oligonucleotides can be prepared by ³²P labeling at the 5' end with polynucleotide kinase (Sambrook et al., *Molecular*
35 *Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory

Press, 1989, Volume 2, pg. 10.59). Radiolabeled oligonucleotides are then contacted with cell or tissue samples suspected of containing B7 message RNAs (and thus B7 proteins), and the samples are washed to remove unbound
5 oligonucleotide. Radioactivity remaining in the sample indicates the presence of bound oligonucleotide, which in turn indicates the presence of nucleic acids complementary to the oligonucleotide, and can be quantitated using a scintillation counter or other routine means. Expression of nucleic acids
10 encoding these proteins is thus detected.

Radiolabeled oligonucleotides of the present invention can also be used to perform autoradiography of tissues to determine the localization, distribution and quantitation of B7 proteins for research, diagnostic or therapeutic purposes.
15 In such studies, tissue sections are treated with radiolabeled oligonucleotide and washed as described above, then exposed to photographic emulsion according to routine autoradiography procedures. The emulsion, when developed, yields an image of silver grains over the regions expressing a B7 gene.
20 Quantitation of the silver grains permits detection of the expression of mRNA molecules encoding these proteins and permits targeting of oligonucleotides to these areas.

Analogous assays for fluorescent detection of expression of B7 nucleic acids can be developed using oligonucleotides
25 of the present invention which are conjugated with fluorescein or other fluorescent tags instead of radiolabeling. Such conjugations are routinely accomplished during solid phase synthesis using fluorescently-labeled amidites or controlled pore glass (CPG) columns. Fluorescein-labeled amidites and
30 CPG are available from, e.g., Glen Research, Sterling VA.

The present invention employs oligonucleotides targeted to nucleic acids encoding B7 proteins and oligonucleotides targeted to nucleic acids encoding such proteins. Kits for detecting the presence or absence of expression of a B7
35 protein may also be prepared. Such kits include an

oligonucleotide targeted to an appropriate gene, i.e., a gene encoding a B7 protein. Appropriate kit and assay formats, such as, e.g., "sandwich" assays, are known in the art and can easily be adapted for use with the oligonucleotides of the invention. Hybridization of the oligonucleotides of the invention with a nucleic acid encoding a B7 protein can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection systems. Kits for detecting the presence or absence of a B7 protein may also be prepared.

In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleotides. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that an oligonucleotide need not be 100% complementary to its target DNA sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target DNA or RNA

molecule interferes with the normal function of the target DNA or RNA to cause a decrease or loss of function, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under
5 conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed.

The formulation of therapeutic compositions and their
10 subsequent administration is believed to be within the skill of those in the art. In general, for therapeutics, a patient in need of such therapy is administered an oligonucleotide in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in doses ranging from 0.01 μ g to 100 g per
15 kg of body weight depending on the age of the patient and the severity of the disorder or disease state being treated. Further, the treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease or disorder, its severity and the overall condition
20 of the patient, and may extend from once daily to once every 20 years. Following treatment, the patient is monitored for changes in his/her condition and for alleviation of the symptoms of the disorder or disease state. The dosage of the oligonucleotide may either be increased in the event the
25 patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disorder or disease state is observed, or if the disorder or disease state has been ablated.

In some cases, it may be more effective to treat a
30 patient with an oligonucleotide of the invention in conjunction with other therapeutic modalities in order to increase the efficacy of a treatment regimen. In the context of the invention, the term "treatment regimen" is meant to encompass therapeutic, palliative and prophylactic modalities.

In a preferred embodiment, the oligonucleotides of the invention are used in conjunction with an anti-inflammatory and/or immunosuppressive agent, preferably one or more antisense oligonucleotides targeted to an intercellular
5 adhesion molecule (ICAM), preferably to ICAM-1. Other anti-inflammatory and/or immunosuppressive agents that may be used in combination with the oligonucleotides of the invention include, but are not limited to, soluble ICAM proteins (e.g., sICAM-1), antibody-toxin conjugates, prednisone,
10 methylprednisolone, azathioprine, cyclophosphamide, cyclosporine, interferons, sympathomimetics, conventional antihistamines (histamine H₁ receptor antagonists, including, for example, brompheniramine maleate, chlorpheniramine maleate, dexchlorpheniramine maleate, tripolidine HCl,
15 carbinoxamine maleate, clemastine fumarate, dimenhydrinate, diphenhydramine HCl, diphenylpyraline HCl, doxylamine succinate, tripeleennamine citrate, tripeleennamine HCl, cyclizine HCl, hydroxyzine HCl, meclizine HCl, methdilazine HCl, promethazine HCl, trimeprazine tartrate, azatadine
20 maleate, cyproheptadine HCl, terfenadine, etc.), histamine H₂ receptor antagonists (e.g., ranitidine). See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 302-336 and 2516-2522). When used with the compounds of the invention, such agents may
25 be used individually, sequentially, or in combination with one or more other such agents.

In another preferred embodiment of the invention, an antisense oligonucleotide targeted to one B7 mRNA species (e.g., B7-1) is used in combination with an antisense
30 oligonucleotide targeted to a second B7 mRNA species (e.g., B7-2) in order to inhibit the costimulatory effect of B7 molecules to a more extensive degree than can be achieved with either oligonucleotide used individually. In a related version of this embodiment, two or more oligonucleotides of
35 the invention, each targeted to an alternatively spliced B7-1

or B7-2 mRNA, are combined with each other in order to inhibit expression of both forms of the alternatively spliced mRNAs. It is known in the art that, depending on the specificity of the modulating agent employed, inhibition of one form of an alternatively spliced mRNA may not result in a sufficient reduction of expression for a given condition to be manifest. Thus, such combinations may, in some instances, be desired to inhibit the expression of a particular B7 gene to an extent necessary to practice one of the methods of the invention.

10 Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μ g to 100 g per kg of body weight, once or more daily, to once every 15 20 years. In the case of an individual known or suspected of being prone to an autoimmune or inflammatory condition, prophylactic effects may be achieved by administration of preventative doses, ranging from 0.01 μ g to 100 g per kg of body weight, once or more daily, to once every 20 years. In 20 like fashion, an individual may be made less susceptible to an inflammatory condition that is expected to occur as a result of some medical treatment, e.g., graft versus host disease resulting from the transplantation of cells, tissue or an organ into the individual.

25 The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and 30 rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular

injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

5 Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated
10 condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may
15 be desirable.

Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

20 Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements
25 of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC₅₀s
30 found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μ g to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years.

The following examples illustrate the invention and are
35 not intended to limit the same. Those skilled in the art will

recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the present
5 invention.

The following examples are provided for illustrative purposes only and are not intended to limit the invention.

EXAMPLES

10 Example 1: Synthesis of Nucleic Acids Oligonucleotides

Oligonucleotides were synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry with oxidation using iodine. β -Cyanoethyldiisopropyl phosphoramidites were purchased from Applied Biosystems
15 (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one-1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed
20 by the capping step.

The 2'-fluoro phosphorothioate oligonucleotides of the invention were synthesized using 5'-dimethoxytrityl-3'-phosphoramidites and prepared as disclosed in U.S. patent application Serial No. 463,358, filed January 11, 1990, and
25 Serial No. 566,977, filed August 13, 1990, which are assigned to the same assignee as the instant application and which are incorporated by reference herein. The 2'-fluoro oligonucleotides were prepared using phosphoramidite chemistry and a slight modification of the standard DNA synthesis
30 protocol: deprotection was effected using methanolic ammonia at room temperature.

The 2'-methoxy (2'-O-methyl) oligonucleotides of the invention were synthesized using 2'-methoxy β -cyanoethyldiisopropyl-phosphoramidites (Chemgenes, Needham MA)

and the standard cycle for unmodified oligonucleotides, except the wait step after pulse delivery of tetrazole and base is increased to 360 seconds. Other 2'-alkoxy oligonucleotides are synthesized by a modification of this method, using appropriate 2'-modified amidites such as those available from Glen Research, Inc., Sterling, VA. The 3'-base used to start the synthesis was a 2'-deoxyribonucleotide. The 2'-O-propyl oligonucleotides of the invention are prepared by a slight modification of this procedure.

10 The 2' methoxyethoxy (2'-O-CH₂CH₂OCH₃) oligonucleotides of the invention were synthesized according to the method of Martin, *Helv. Chim. Acta* 1995, 78, 486. For ease of synthesis, the last nucleotide was a deoxynucleotide. All 2'-O-CH₂CH₂OCH₃-cytosines were 5-methyl cytosines, which were
15 synthesized according to the following procedures.

Synthesis of 5-Methyl cytosine monomers:

2,2'-Anhydro[1-(β-D-arabinofuranosyl)-5-methyluridine]

5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenyl-
20 carbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced
25 pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted
30 and the gum was dried in a vacuum oven (60EC at 1 mm Hg for 24 h) to give a solid which was crushed to a light tan powder (57 g, 85% crude yield). The material was used as is for further reactions.

2'-O-Methoxyethyl-5-methyluridine

2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160EC. After heating for 48 hours at 155-160EC, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH₃CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH₂Cl₂/acetone/MeOH (20:5:3) containing 0.5% Et₃NH. The residue was dissolved in CH₂Cl₂ (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH₃CN (200 mL). The residue was dissolved in CHCl₃ (1.5 L) and extracted with 2x500 mL of saturated NaHCO₃ and 2x500 mL of saturated NaCl. The organic phase was dried over Na₂SO₄, filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/Hexane/Acetone (5:5:1) containing 0.5% Et₃NH. The pure

fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by tlc by first quenching the tlc sample with the addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL) was added and the mixture evaporated at 35EC. The residue was dissolved in CHCl_3 (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water layers were back extracted with 200 mL of CHCl_3 . The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane(4:1). Pure product fractions were evaporated to yield 96 g (84%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to -5EC and stirred for 0.5 h using an overhead stirrer. POCl_3 was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10EC, and the resulting mixture stirred for an additional 2 hours. The first solution was added to the later solution dropwise, over

a 45 minute period. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1x300 mL of NaHCO₃ and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

10 A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH₄OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x200 mL). The residue
15 was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH₃ gas was added and the vessel heated to 100EC for 2 hours (tlc showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc
20 (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

N⁴-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

25 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, tlc showed the reaction to be approximately 95% complete. The solvent was evaporated and
30 the residue azeotroped with MeOH (200 mL). The residue was dissolved in CHCl₃ (700 mL) and extracted with saturated NaHCO₃

(2x300 mL) and saturated NaCl (2x300 mL), dried over MgSO_4 and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure
5 product fractions were evaporated to give 90 g (90%) of the title compound.

N^4 -Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

N^4 -Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-
10 methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (tlc showed the
15 reaction to be 95% complete). The reaction mixture was extracted with saturated NaHCO_3 (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over MgSO_4 and concentrated. The residue obtained was chromatographed on a
20 1.5 kg silica column using EtOAc/Hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites:

25 **2'-(Dimethylaminooxyethoxy) nucleoside amidites**

2'-(Dimethylaminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside
30 amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected

with a benzoyl moiety in the case of adenosine and cytidine and with isobutyryl in the case of guanosine.

5'-O-*tert*-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine

O²-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0g, 0.416 mmol), dimethylaminopyridine (0.66g, 0.013eq, 0.0054mmol) were dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. *tert*-Butyldiphenylchlorosilane (125.8g, 119.0mL, 1.1eq, 0.458mmol) was added in one portion. The reaction was stirred for 16 h at ambient temperature. TLC (Rf 0.22, ethyl acetate) indicated a complete reaction. The solution was concentrated under reduced pressure to a thick oil. This was partitioned between dichloromethane (1 L) and saturated sodium bicarbonate (2x1 L) and brine (1 L). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil was dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600mL) and the solution was cooled to -10°C. The resulting crystalline product was collected by filtration, washed with ethyl ether (3x200 mL) and dried (40°C, 1mm Hg, 24 h) to 149g (74.8%) of white solid. TLC and NMR were consistent with pure product.

5'-O-*tert*-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

In a 2 L stainless steel, unstirred pressure reactor was added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622 mL). In the fume hood and with manual stirring, ethylene glycol (350 mL, excess) was added cautiously at first until the evolution of hydrogen gas subsided. 5'-O-*tert*-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine (149 g, 0.311 mol) and sodium bicarbonate (0.074 g, 0.003 eq) were added with manual stirring. The reactor was sealed and heated in an oil bath until an internal temperature of 160°C was reached and then

maintained for 16 h (pressure < 100 psig). The reaction vessel was cooled to ambient and opened. TLC (Rf 0.67 for desired product and Rf 0.82 for are-T side product, ethyl acetate) indicated about 70% conversion to the product. In order to avoid additional side product formation, the reaction was stopped, concentrated under reduced pressure (10 to 1mm Hg) in a warm water bath (40-100°C) with the more extreme conditions used to remove the ethylene glycol. [Alternatively, once the low boiling solvent is gone, the remaining solution can be partitioned between ethyl acetate and water. The product will be in the organic phase.] The residue was purified by column chromatography (2kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions were combined, stripped and dried to product as a white crisp foam (84g, 50%), contaminated starting material (17.4g) and pure reusable starting material 20g. The yield based on starting material less pure recovered starting material was 58%. TLC and NMR were consistent with 99% pure product.

20

2'-O-([2-phthalimidoxy)ethyl]-5'-*t*-butyldiphenylsilyl-5-methyluridine

5'-O-*tert*-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine (20g, 36.98mmol) was mixed with triphenylphosphine (11.63g, 44.36mmol) and N-hydroxyphthalimide (7.24g, 44.36mmol). It was then dried over P₂O₅ under high vacuum for two days at 40°C. The reaction mixture was flushed with argon and dry THF (369.8mL, Aldrich, sure seal bottle) was added to get a clear solution. Diethyl-azodicarboxylate (6.98mL, 44.36mmol) was added dropwise to the reaction mixture. The rate of addition is maintained such that resulting deep red coloration is just discharged before adding the next drop. After the addition was complete, the reaction was stirred for 4 hrs. By that time TLC showed the completion of the reaction

(ethylacetate:hexane, 60:40). The solvent was evaporated in vacuum. Residue obtained was placed on a flash column and eluted with ethyl acetate:hexane (60:40), to get 2'-O-([2-phthalimidooxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine as white foam (21.819 g, 86%).

5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine

2'-O-([2-phthalimidooxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine (3.1g, 4.5mmol) was dissolved in dry CH₂Cl₂ (4.5mL) and methylhydrazine (300mL, 4.64mmol) was added dropwise at -10°C to 0°C. After 1 h the mixture was filtered, the filtrate was washed with ice cold CH₂Cl₂ and the combined organic phase was washed with water, brine and dried over anhydrous Na₂SO₄. The solution was concentrated to get 2'-O-(aminooxyethyl) thymidine, which was then dissolved in MeOH (67.5mL). To this formaldehyde (20% aqueous solution, w/w, 1.1 eq.) was added and the resulting mixture was stirred for 1 h. Solvent was removed under vacuum; residue chromatographed to get 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-methyluridine as white foam (1.95 g, 78%).

5'-O-tert-Butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-

5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine (1.77g, 3.12mmol) was dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6mL). Sodium cyanoborohydride (0.39g, 6.13mmol) was added to this solution at 10°C under inert atmosphere. The reaction mixture was stirred for 10 minutes at 10°C. After that the reaction vessel was removed from the ice bath and stirred at room temperature for 2 h, the reaction monitored by TLC (5% MeOH in CH₂Cl₂). Aqueous NaHCO₃ solution

(5%, 10mL) was added and extracted with ethyl acetate (2x20mL). Ethyl acetate phase was dried over anhydrous Na₂SO₄, evaporated to dryness. Residue was dissolved in a solution of 1M PPTS in MeOH (30.6mL). Formaldehyde (20% w/w, 30mL, 5 3.37mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. Reaction mixture cooled to 10°C in an ice bath, sodium cyanoborohydride (0.39g, 6.13mmol) was added and reaction mixture stirred at 10°C for 10 minutes. After 10 minutes, the reaction mixture was removed from the 10 ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% NaHCO₃ (25mL) solution was added and extracted with ethyl acetate (2x25mL). Ethyl acetate layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue obtained was purified by flash column 15 chromatography and eluted with 5% MeOH in CH₂Cl₂ to get 5'-O-tert-butyl diphenylsilyl-2'-O-[N,N-dimethylaminoxyethyl]-5-methyluridine as a white foam (14.6g, 80%).

2'-O-(dimethylaminoxyethyl)-5-methyluridine

20 Triethylamine trihydrofluoride (3.91mL, 24.0mmol) was dissolved in dry THF and triethylamine (1.67mL, 12mmol, dry, kept over KOH). This mixture of triethylamine-2HF was then added to 5'-O-tert-butyl diphenylsilyl-2'-O-[N,N-dimethylaminoxyethyl]-5-methyluridine (1.40g, 2.4mmol) and 25 stirred at room temperature for 24 hrs. Reaction was monitored by TLC (5% MeOH in CH₂Cl₂). Solvent was removed under vacuum and the residue placed on a flash column and eluted with 10% MeOH in CH₂Cl₂ to get 2'-O-(dimethylaminoxyethyl)-5-methyluridine (766mg, 92.5%).

30 5'-O-DMT-2'-O-(dimethylaminoxyethyl)-5-methyluridine

2'-O-(dimethylaminoxyethyl)-5-methyluridine (750mg,

2.17mmol) was dried over P_2O_5 under high vacuum overnight at 40°C. It was then co-evaporated with anhydrous pyridine (20mL). The residue obtained was dissolved in pyridine (11mL) under argon atmosphere. 4-dimethylaminopyridine (26.5mg, 2.60mmol), 4,4'-dimethoxytrityl chloride (880mg, 2.60mmol) was added to the mixture and the reaction mixture was stirred at room temperature until all of the starting material disappeared. Pyridine was removed under vacuum and the residue chromatographed and eluted with 10% MeOH in CH_2Cl_2 (containing a few drops of pyridine) to get 5'-O-DMT-2'-O-(dimethylamino-oxyethyl)-5-methyluridine (1.13g, 80%).

5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

5'-O-DMT-2'-O-(dimethylaminoxyethyl)-5-methyluridine (1.08g, 1.67mmol) was co-evaporated with toluene (20mL). To the residue N,N-diisopropylamine tetrazonide (0.29g, 1.67mmol) was added and dried over P_2O_5 under high vacuum overnight at 40°C. Then the reaction mixture was dissolved in anhydrous acetonitrile (8.4mL) and 2-cyanoethyl-N,N,N1,N1-tetraisopropylphosphoramidite (2.12mL, 6.08mmol) was added. The reaction mixture was stirred at ambient temperature for 4 hrs under inert atmosphere. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 1:1). The solvent was evaporated, then the residue was dissolved in ethyl acetate (70mL) and washed with 5% aqueous $NaHCO_3$ (40mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated. Residue obtained was chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam (1.04g, 74.9%).

2'-(Aminooxyethoxy) nucleoside amidites

2'-(Aminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(aminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and thymidine nucleoside amidites are prepared similarly.

N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

The 2'-O-aminooxyethyl guanosine analog may be obtained by selective 2'-O-alkylation of diaminopurine riboside. Multigram quantities of diaminopurine riboside may be purchased from Schering AG (Berlin) to provide 2'-O-(2-ethylacetyl) diaminopurine riboside along with a minor amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl) diaminopurine riboside may be resolved and converted to 2'-O-(2-ethylacetyl)guanosine by treatment with adenosine deaminase. (PCT WO94/02501). Standard protection procedures should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine which may be reduced to provide 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be displaced by N-hydroxyphthalimide via a Mitsunobu reaction, and the protected nucleoside may phosphitylated as usual to yield 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite].

2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites

2'-dimethylaminoethoxyethoxy nucleoside amidites (also known in the art as 2'-O-dimethylaminoethoxyethyl, i.e., 2'-O-CH₂-O-

$\text{CH}_2\text{-N}(\text{CH}_2)_2$, or 2'-DMAEOE nucleoside amidites) are prepared as follows. Other nucleoside amidites are prepared similarly.

2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]-5-methyl uridine

5 2[2-(Dimethylamino)ethoxy]ethanol (Aldrich, 6.66 g, 50 mmol) is slowly added to a solution of borane in tetrahydrofuran (1 M, 10 mL, 10 mmol) with stirring in a 100 mL bomb. Hydrogen gas evolves as the solid dissolves. 02-,2'-anhydro-5-methyluridine (1.2 g, 5 mmol), and sodium
10 bicarbonate (2.5 mg) are added and the bomb is sealed, placed in an oil bath and heated to 155 C for 26 hours. The bomb is cooled to room temperature and opened. The crude solution is concentrated and the residue partitioned between water (200 mL) and hexanes (200 mL). The excess phenol is extracted into
15 the hexane layer. The aqueous layer is extracted with ethyl acetate (3x200 mL) and the combined organic layers are washed once with water, dried over anhydrous sodium sulfate and concentrated. The residue is columned on silica gel using methanol/methylene chloride 1:20 (which has 2% triethylamine)
20 as the eluent. As the column fractions are concentrated a colorless solid forms which is collected to give the title compound as a white solid.

5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]-5-methyl uridine

25 To 0.5 g (1.3 mmol) of 2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]-5-methyl uridine in anhydrous pyridine (8 mL), triethylamine (0.36 mL) and dimethoxytrityl chloride (DMT-Cl, 0.87 g, 2 eq.) are added and stirred for 1 hour. The reaction mixture is poured into water (200 mL) and extracted with
30 CH_2Cl_2 (2x200 mL). The combined CH_2Cl_2 layers are washed with saturated NaHCO_3 solution, followed by saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the

solvent followed by silica gel chromatography using MeOH:CH₂Cl₂:Et₃N (20:1, v/v, with 1% triethylamine) gives the title compound.

5'-O-Dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]]-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite

Diisopropylaminotetrazolide (0.6 g) and 2-cyanoethoxy-N,N-diisopropyl phosphoramidite (1.1 mL, 2 eq.) are added to a solution of 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]]-5-methyluridine (2.17 g, 3 mmol) dissolved in CH₂Cl₂ (20 mL) under an atmosphere of argon. The reaction mixture is stirred overnight and the solvent evaporated. The resulting residue is purified by silica gel flash column chromatography with ethyl acetate as the eluent to give the title compound.

Purification:

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, pH 7.0. Oligodeoxynucleotides and their phosphorothioate analogs were judged from electrophoresis to be greater than 80% full length material.

B7 Antisense Oligonucleotides

A series of oligonucleotides with sequences designed to hybridize to the published human B7-1 (hB7-1) and murine (mB7-1) mRNA sequences (Freeman et al., *J. Immunol.*, 1989, 143, 2714, and Freeman et al., *J. Exp. Med.*, 1991, 174, 625 respectively). The sequences of and modifications to these

oligonucleotides, and the location of each of their target sites on the hB7-1 mRNA, are given in Tables 1 and 2. Similarly, a series of oligonucleotides with sequences designed to hybridize to the human B7-2 (hB7-2) and murine B7-2 (mB7-2) mRNA published sequences (respectively, Azuma et al., *Nature*, 1993, 366, 76; Chen et al., *J. Immunol.*, 1994, 152, 4929) were synthesized. The sequences of and modifications to these oligonucleotides and the location of each of their target sites on the hB7-2 mRNA are described in Tables 3 and 4. Antisense oligonucleotides targeted to ICAM-1, including ISIS 2302 (SEQ ID NO: 17), have been described in U.S. Patent No. 5,514,788, which issued May 7, 1996, hereby incorporated by reference. ISIS 1082 (SEQ ID NO: 102) and ISIS 3082 (SEQ ID NO: 101) have been previously described (Stepkowski et al., *J. Immunol.*, 1994, 153, 5336).

Subsequent to their initial cloning, alternative splicing events of B7 transcripts have been reported. The reported alternative splicing for B7-1 is relatively simple, in that it results in messages extended 5' relative to the 5' terminus of the human and murine B7-1 cDNA sequences originally reported (Borriello et al., *J. Immunol.*, 1994, 153, 5038; Inobe et al., *J. Immunol.*, 1996, 157, 588). In order to retain the numbering of the B7-1 sequences found in the references initially reporting B7-1 sequences, positions within these 5' extensions of the initially reported sequences have been given negative numbers (beginning with position -1, the most 3' base of the 5' extension) in Tables 1 and 2. The processing of murine B7-2 transcripts is considerably more complex than that so far reported for B7-1; for example, at least five distinct murine B7-2 mRNAs, and at least two distinct human B7-2 mRNAs, can be produced by alternative splicing events (Borriello et al., *J. Immunol.*, 1995, 155, 5490; Freeman et al., WO 95/03408, published February 2, 1995; see also Jellis et al., *Immunogenet.*, 1995, 42, 85). The

nature of these splicing events is such that different 5' exons are used to produce distinct B7-2 mRNAs, each of which has a unique 5' sequence but which share a 3' portion consisting of some or all of the B7-2 sequence initially reported. As a result, positions within the 5' extensions of B7-2 messages cannot be uniquely related to a position within the sequence initially reported. Accordingly, in Table 3, a different set of coordinates (corresponding to those of SEQ ID NO: 1 of WO 95/03408) and, in Table 4, the exon number (as given in Borriello et al., *J. Immunol.*, 1995, 155, 5490) is used to specify the location of targeted sequences which are not included in the initially reported B7-2 sequence. Furthermore, although these 5' extended messages contain potential in-frame start codons upstream from the ones indicated in the initially published sequences, for simplicity's sake, such additional potential start codons are not indicated in the description of target sites in Tables 1-4.

In Tables 1-4, the following abbreviations are used: UTR, untranslated region; ORF, open reading frame; tIR, translation initiation region; tTR, translation termination region; FITC, fluorescein isothiocyanate. Chemical modifications are indicated as follows. Residues having 2' fluoro (2'F), 2'-methoxy (2'MO) or 2'-methoxyethoxy (2'ME) modification are emboldened, with the type of modification being indicated by the respective abbreviations. Unless otherwise indicated, interresidue linkages are phosphodiester linkages; phosphorothioate linkages are indicated by an "S" in the superscript position (e.g., T^SA). Target positions are numbered according to Freeman et al., *J. Immunol.*, 1989, 143:2714 (human B7-1 cDNA sequence; Table 1), Freeman et al., *J. Exp. Med.*, 1991, 174, 625 (murine B7-1 cDNA sequence; Table 2), Azuma et al., *Nature*, 1993, 366:76 (human B7-2 cDNA

sequence; Table 3) and Chen *et al.*, *J. Immunol.*, 1994, 152:4929 (murine B7-2 cDNA sequence; Table 4). Nucleotide base codes are as given in 37 C.F.R. §1.822(b)(1).

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TABLE 1
Sequences of Oligonucleotides Targeted to Human B7-1 mRNA

ISIS #	Target Position; Site (and/or Description)	Oligonucleotide Sequence(5'->3') and Chemical Modifications	SEQ ID NO:
13797	0053-0072; 5' UTR	G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s A ^s C ^s T ^s T ^s C ^s T ^s G ^s A	22
13798	0132-0151; 5' UTR	G ^s G ^s T ^s C ^s T ^s C ^s T ^s C ^s A ^s A ^s A ^s G ^s G ^s T ^s T ^s G ^s T ^s G ^s G ^s A	23
13799	0138-0157; 5' UTR	G ^s T ^s T ^s C ^s C ^s T ^s T ^s G ^s G ^s T ^s C ^s T ^s C ^s C ^s A ^s A ^s A ^s G ^s G ^s T	24
13800	0158-0177; 5' UTR	A ^s C ^s A ^s C ^s A ^s C ^s A ^s G ^s A ^s G ^s A ^s T ^s T ^s G ^s A ^s G ^s G ^s T	25
13801	0193-0212; 5' UTR	G ^s C ^s T ^s C ^s A ^s C ^s G ^s T ^s A ^s G ^s A ^s A ^s G ^s A ^s C ^s C ^s C ^s T ^s C ^s C	26
13802	0217-0236; 5' UTR	G ^s G ^s C ^s A ^s G ^s G ^s C ^s T ^s G ^s A ^s T ^s G ^s A ^s C ^s A ^s A ^s T ^s C ^s C	27
13803	0226-0245; 5' UTR	T ^s G ^s C ^s A ^s A ^s A ^s C ^s A ^s C ^s A ^s G ^s C ^s A ^s G ^s C ^s A ^s G ^s G ^s C ^s T ^s G ^s A	28
13804	0246-0265; 5' UTR	A ^s G ^s A ^s C ^s C ^s A ^s G ^s G ^s G ^s C ^s A ^s C ^s T ^s T ^s C ^s C ^s A ^s G ^s G	29
13805	0320-0339; tIR	C ^s C ^s T ^s G ^s C ^s C ^s T ^s C ^s C ^s G ^s T ^s C ^s G ^s T ^s G ^s T ^s G ^s C ^s C ^s C	30
13806	0380-0399; 5' ORF	G ^s A ^s C ^s C ^s A ^s G ^s C ^s C ^s A ^s G ^s C ^s A ^s C ^s A ^s A ^s G ^s A ^s G ^s C	31
13807	0450-0469; 5' ORF	C ^s C ^s A ^s C ^s A ^s G ^s G ^s A ^s C ^s A ^s G ^s C ^s G ^s T ^s T ^s G ^s C ^s C ^s A ^s C	32
13808	0568-0587; 5' ORF	C ^s C ^s G ^s T ^s C ^s T ^s T ^s G ^s T ^s A ^s C ^s T ^s C ^s G ^s G ^s C ^s C	33
13809	0634-0653; central ORF	G ^s C ^s C ^s C ^s T ^s C ^s G ^s T ^s C ^s A ^s G ^s A ^s T ^s G ^s G ^s C ^s G ^s C ^s A	51

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13810	0829-0848; central ORF	C ^s C ^s A ^s A ^s C ^s C ^s A ^s G ^s G ^s A ^s G ^s A ^s G ^s G ^s T ^s G ^s A ^s G ^s G ^s C	34
13811	1102-1121; 3' ORF	G ^s G ^s C ^s A ^s A ^s A ^s G ^s C ^s A ^s G ^s T ^s A ^s G ^s G ^s T ^s C ^s A ^s G ^s G ^s C	35
13812	1254-1273; 3'-UTR	G ^s C ^s C ^s T ^s C ^s A ^s T ^s G ^s A ^s T ^s C ^s C ^s C ^s C ^s A ^s C ^s G ^s A ^s T ^s C	36
13872	(scrambled # 13812)	A ^s G ^s T ^s C ^s C ^s T ^s A ^s C ^s T ^s A ^s C ^s C ^s A ^s G ^s C ^s C ^s G ^s C ^s C ^s T	52
12361	0056-0075; 5' UTR	T ^s C ^s A ^s G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s A ^s C ^s T ^s T ^s C	38
12348	0056-0075; 5' UTR	T C A G G T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s C A C T T C (2' ME)	38
12473	0056-0075; 5' UTR	T ^s C ^s A ^s G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s C A C T T C (2' FL)	38
12362	0143-0162; 5' UTR	A ^s G ^s G ^s G ^s T ^s G ^s T ^s T ^s C ^s C ^s T ^s G ^s G ^s T ^s C ^s T ^s C ^s A	39
12349	0143-0162; 5' UTR	A G G G T G ^s T ^s T ^s C ^s C ^s T ^s G ^s G ^s T ^s C T C C A (2' ME)	39
12474	0143-0162; 5' UTR	A ^s G ^s G ^s G ^s T ^s G ^s T ^s T ^s C ^s C ^s T ^s G ^s G ^s T ^s C ^s T ^s C ^s A (2' FL)	39
12363	0315-0334; tIR	C ^s T ^s C ^s C ^s G ^s T ^s G ^s T ^s G ^s T ^s G ^s C ^s C ^s A ^s T ^s G ^s G ^s C	40
12350	0315-0334; tIR	C T C C G T ^s G ^s T ^s G ^s T ^s G ^s G ^s C C C A T G G C (2' ME)	40
12475	0315-0334; tIR	C ^s T ^s C ^s C ^s G ^s T ^s G ^s T ^s G ^s G ^s C ^s C ^s C ^s A ^s T ^s G ^s G ^s C (2' FL)	40
12364	0334-0353; 5' ORF	G ^s G ^s A ^s T ^s G ^s G ^s T ^s G ^s A ^s T ^s G ^s T ^s C ^s C ^s T ^s G ^s C	41

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12351	0334-0353; 5' ORF	G G A T G G T ^s G A ^s T ^s G T ^s T ^s C C C T G C C (2' ME)	41
12476	0334-0353; 5' ORF	G ^s G ^s A ^s T ^s G ^s T ^s G A ^s T ^s G T ^s T ^s C C C T G C C (2' F1)	41
12365	0387-0406; 5' ORF	T ^s G ^s A ^s G ^s A ^s A ^s G ^s A ^s C ^s A ^s G ^s C ^s A ^s G ^s C ^s A ^s C (2' ME)	42
12352	0387-0406; 5' ORF	T G A G A A ^s G ^s A ^s C ^s A ^s G ^s C ^s A G C A C (2' ME)	42
12477	0387-0406; 5' ORF	T ^s G ^s A ^s G ^s A ^s A ^s G ^s A ^s C ^s A ^s G ^s C ^s A ^s G ^s C ^s A ^s C (2' F1)	42
12366	0621-0640; central ORF	G ^s G ^s C ^s C ^s G ^s A ^s G ^s A ^s G ^s C ^s A ^s G ^s A ^s T ^s C ^s A ^s C (2' ME)	43
12353	0621-0640; central ORF	G G G C G C ^s A ^s G ^s A ^s G ^s C ^s A ^s G G A T C A C (2' ME)	43
12478	0621-0640; central ORF	G ^s G ^s C ^s C ^s G ^s A ^s G ^s A ^s G ^s C ^s A ^s G ^s A ^s T ^s C ^s A ^s C (2' F1)	43
12367	1042-1061; 3' ORF	G ^s G ^s C ^s C ^s A ^s G ^s A ^s T ^s G ^s G ^s A ^s G ^s C ^s A ^s G ^s G ^s T (2' ME)	44
12354	1042-1061; 3' ORF	G G C C A ^s G ^s A ^s T ^s G ^s G ^s A G C A G G T (2' ME)	44
12479	1042-1061; 3' ORF	G ^s G ^s C ^s C ^s A ^s G ^s A ^s T ^s G ^s G ^s A ^s G ^s C ^s A ^s G ^s T (2' F1)	44
12368	1069-1088; tTR	A ^s G ^s G ^s C ^s C ^s G ^s T ^s A ^s C ^s A ^s C ^s T ^s T ^s T ^s C ^s C ^s T ^s T ^s C (2' ME)	45
12355	1069-1088; tTR	A G G C G T ^s A ^s C ^s A ^s C ^s T ^s T ^s T ^s C C C T C (2' ME)	45

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TABLE 2

Sequences of Oligonucleotides Targeted to Murine B7-1 mRNA

ISIS #	Target Position; Site	Oligonucleotide Sequence (5'→3') and Chemical Modifications	SEQ ID NO:
14419	0009-0028; 5' UTR	A ^s G ^s T ^s A ^s A ^s G ^s A ^s G ^s T ^s C ^s T ^s A ^s T ^s T ^s G ^s A ^s G ^s G ^s T ^s A	53
14420	0041-0060; 5' UTR	G ^s G ^s T ^s T ^s G ^s A ^s G ^s T ^s T ^s T ^s C ^s A ^s C ^s A ^s A ^s C ^s C ^s T ^s G ^s A	54
14421	0071-0091; 5' UTR	G ^s T ^s C ^s A ^s C ^s A ^s G ^s A ^s A ^s T ^s G ^s G ^s A ^s C ^s A ^s G ^s A ^s G	55
14422	0109-0128; 5' UTR	G ^s G ^s C ^s A ^s T ^s C ^s C ^s A ^s C ^s C ^s G ^s C ^s G ^s A ^s G ^s A ^s T ^s G ^s C	56
14423	0114-0133; 5' UTR	T ^s G ^s G ^s A ^s T ^s G ^s G ^s C ^s A ^s T ^s C ^s C ^s A ^s C ^s C ^s G ^s G ^s C ^s A	57
14424	0168-0187; 5' UTR	A ^s G ^s G ^s C ^s A ^s C ^s C ^s T ^s C ^s C ^s T ^s A ^s G ^s G ^s C ^s T ^s C ^s A ^s C ^s A	58
14425	0181-0200; 5' UTR	G ^s C ^s C ^s A ^s A ^s T ^s G ^s G ^s A ^s G ^s C ^s T ^s T ^s A ^s G ^s G ^s C ^s A ^s C ^s C	59
14426	0208-0217; 5' UTR	C ^s A ^s T ^s G ^s A ^s T ^s G ^s G ^s G ^s A ^s A ^s G ^s C ^s C ^s A ^s G ^s G ^s A	60
14427	0242-0261; tIR	A ^s A ^s T ^s T ^s G ^s C ^s A ^s A ^s G ^s C ^s C ^s A ^s T ^s A ^s G ^s C ^s T ^s T ^s C ^s A	61
14428	0393-0412; 5' ORF	C ^s G ^s G ^s C ^s A ^s A ^s G ^s G ^s C ^s A ^s G ^s C ^s A ^s T ^s A ^s C ^s C ^s T ^s T	62
14909	0478-0497; 5' ORF	C ^s C ^s C ^s A ^s G ^s C ^s A ^s T ^s G ^s A ^s C ^s A ^s C ^s A ^s G ^s A ^s G ^s C ^s A	63
14910	0569-0588; central ORF	G ^s G ^s T ^s C ^s T ^s G ^s A ^s A ^s G ^s A ^s G ^s A ^s C ^s C ^s A ^s G ^s C ^s C ^s C	64
14911	0745-0764; central ORF	T ^s G ^s G ^s G ^s A ^s A ^s C ^s C ^s C ^s C ^s G ^s G ^s A ^s A ^s G ^s C ^s A	65
14912	0750-0769; central ORF	G ^s G ^s C ^s T ^s T ^s G ^s G ^s G ^s A ^s A ^s C ^s C ^s C ^s C ^s C ^s G ^s G ^s A	66

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14913	0825-0844; 3' ORF	T ^s C ^s A ^s G ^s A ^s T ^s T ^s C ^s A ^s G ^s G ^s A ^s T ^s C ^s C ^s T ^s G ^s G ^s A	67
14914	0932-0951; 3' ORF	C ^s C ^s A ^s G ^s G ^s T ^s G ^s A ^s A ^s G ^s T ^s C ^s C ^s T ^s C ^s T ^s G ^s A ^s C	68
14915	1001-1020; 3' ORF	C ^s T ^s G ^s C ^s G ^s C ^s C ^s G ^s A ^s T ^s C ^s C ^s T ^s G ^s C ^s C ^s C ^s A	69
14916	1125-1144; tTR	C ^s A ^s G ^s G ^s C ^s C ^s C ^s A ^s A ^s G ^s G ^s T ^s A ^s A ^s G ^s G ^s C ^s T ^s G	70
14917	1229-1248; 3' UTR	T ^s C ^s A ^s G ^s C ^s T ^s A ^s G ^s C ^s A ^s C ^s G ^s T ^s G ^s C ^s T ^s G ^s A	71
14918	1329-1348; 3' UTR	G ^s G ^s C ^s C ^s A ^s G ^s C ^s A ^s A ^s C ^s T ^s T ^s G ^s C ^s C ^s G ^s T	72
14919	1377-1393; 3' UTR	C ^s C ^s A ^s C ^s C ^s A ^s C ^s A ^s G ^s T ^s G ^s G ^s C ^s T ^s C ^s A ^s G ^s C ^s C	73
12912	-0067 to -0049; 5' UTR	G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s G ^s C ^s A ^s T ^s C ^s T ^s A	74
12913	-0067 to -0047; 5' UTR	G ^s T ^s G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s G ^s C ^s A ^s T ^s C ^s T ^s A	75
13496	-0067 to -0047; 5' UTR	G ^s T ^s G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s G ^s C ^s A ^s T ^s C ^s T ^s A	75
13497	-0067 to -0047; 5' UTR	G T G G C C A T G A G G G C A A T C T A A	75

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oligonucleotides, and the location of each of their target sites on the hB7-1 mRNA, are given in Tables 1 and 2. Similarly, a series of oligonucleotides with sequences designed to hybridize to the human B7-2 (hB7-2) and murine B7-2 (mB7-2) mRNA published sequences (respectively, Azuma et al., *Nature*, 1993, 366, 76; Chen et al., *J. Immunol.*, 1994, 152, 4929) were synthesized. The sequences of and modifications to these oligonucleotides and the location of each of their target sites on the hB7-2 mRNA are described in Tables 3 and 4. Antisense oligonucleotides targeted to ICAM-1, including ISIS 2302 (SEQ ID NO: 17), have been described in U.S. Patent No. 5,514,788, which issued May 7, 1996, hereby incorporated by reference. ISIS 1082 (SEQ ID NO: 102) and ISIS 3082 (SEQ ID NO: 101) have been previously described (Stepkowski et al., *J. Immunol.*, 1994, 153, 5336).

Subsequent to their initial cloning, alternative splicing events of B7 transcripts have been reported. The reported alternative splicing for B7-1 is relatively simple, in that it results in messages extended 5' relative to the 5' terminus of the human and murine B7-1 cDNA sequences originally reported (Borriello et al., *J. Immunol.*, 1994, 153, 5038; Inobe et al., *J. Immunol.*, 1996, 157, 588). In order to retain the numbering of the B7-1 sequences found in the references initially reporting B7-1 sequences, positions within these 5' extensions of the initially reported sequences have been given negative numbers (beginning with position -1, the most 3' base of the 5' extension) in Tables 1 and 2. The processing of murine B7-2 transcripts is considerably more complex than that so far reported for B7-1; for example, at least five distinct murine B7-2 mRNAs, and at least two distinct human B7-2 mRNAs, can be produced by alternative splicing events (Borriello et al., *J. Immunol.*, 1995, 155, 5490; Freeman et al., WO 95/03408, published February 2, 1995; see also Jellis et al., *Immunogenet.*, 1995, 42, 85). The

nature of these splicing events is such that different 5' exons are used to produce distinct B7-2 mRNAs, each of which has a unique 5' sequence but which share a 3' portion consisting of some or all of the B7-2 sequence initially reported. As a result, positions within the 5' extensions of B7-2 messages cannot be uniquely related to a position within the sequence initially reported. Accordingly, in Table 3, a different set of coordinates (corresponding to those of SEQ ID NO: 1 of WO 95/03408) and, in Table 4, the exon number (as given in Borriello et al., *J. Immunol.*, 1995, 155, 5490) is used to specify the location of targeted sequences which are not included in the initially reported B7-2 sequence. Furthermore, although these 5' extended messages contain potential in-frame start codons upstream from the ones indicated in the initially published sequences, for simplicity's sake, such additional potential start codons are not indicated in the description of target sites in Tables 1-4.

In Tables 1-4, the following abbreviations are used: UTR, untranslated region; ORF, open reading frame; tIR, translation initiation region; tTR, translation termination region; FITC, fluorescein isothiocyanate. Chemical modifications are indicated as follows. Residues having 2' fluoro (2'F), 2'-methoxy (2'MO) or 2'-methoxyethoxy (2'ME) modification are emboldened, with the type of modification being indicated by the respective abbreviations. Unless otherwise indicated, interresidue linkages are phosphodiester linkages; phosphorothioate linkages are indicated by an "S" in the superscript position (e.g., T^SA). Target positions are numbered according to Freeman et al., *J. Immunol.*, 1989, 143:2714 (human B7-1 cDNA sequence; Table 1), Freeman et al., *J. Exp. Med.*, 1991, 174, 625 (murine B7-1 cDNA sequence; Table 2), Azuma et al., *Nature*, 1993, 366:76 (human B7-2 cDNA

sequence; Table 3) and Chen et al., *J. Immunol.*, 1994, 152:4929 (murine B7-2 cDNA sequence; Table 4). Nucleotide base codes are as given in 37 C.F.R. §1.822(b)(1).

TABLE 1
Sequences of Oligonucleotides Targeted to Human B7-1 mRNA

ISIS #	Target Position; Site (and/or Description)	Oligonucleotide Sequence (5'→3') and Chemical Modifications	SEQ ID NO:
13797	0053-0072; 5' UTR	G ^s G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s A ^s C ^s T ^s T ^s C ^s T ^s G ^s A	22
13798	0132-0151; 5' UTR	G ^s G ^s G ^s T ^s C ^s T ^s C ^s A ^s A ^s A ^s G ^s T ^s T ^s G ^s T ^s G ^s A	23
13799	0138-0157; 5' UTR	G ^s T ^s T ^s C ^s C ^s T ^s G ^s G ^s T ^s C ^s T ^s C ^s A ^s A ^s G ^s G ^s T	24
13800	0158-0177; 5' UTR	A ^s C ^s A ^s C ^s A ^s C ^s A ^s G ^s A ^s G ^s A ^s T ^s T ^s G ^s A ^s G ^s G ^s T	25
13801	0193-0212; 5' UTR	G ^s C ^s T ^s C ^s A ^s C ^s G ^s T ^s A ^s G ^s A ^s A ^s G ^s A ^s C ^s C ^s T ^s C ^s C	26
13802	0217-0236; 5' UTR	G ^s G ^s C ^s A ^s G ^s G ^s C ^s T ^s G ^s A ^s T ^s G ^s A ^s C ^s A ^s T ^s C ^s C	27
13803	0226-0245; 5' UTR	T ^s G ^s C ^s A ^s A ^s A ^s C ^s A ^s G ^s G ^s A ^s G ^s C ^s A ^s G ^s G ^s C ^s T ^s G ^s A	28
13804	0246-0265; 5' UTR	A ^s G ^s A ^s C ^s C ^s A ^s G ^s G ^s G ^s C ^s A ^s C ^s T ^s T ^s C ^s C ^s A ^s G ^s G	29
13805	0320-0339; tIR	C ^s C ^s T ^s G ^s C ^s C ^s T ^s C ^s C ^s G ^s T ^s G ^s T ^s G ^s T ^s G ^s C ^s C ^s C	30
13806	0380-0399; 5' ORF	G ^s A ^s C ^s C ^s A ^s G ^s C ^s C ^s A ^s G ^s C ^s A ^s C ^s C ^s A ^s G ^s A ^s G ^s C	31
13807	0450-0469; 5' ORF	C ^s C ^s A ^s C ^s A ^s G ^s G ^s A ^s C ^s A ^s G ^s C ^s G ^s T ^s T ^s G ^s C ^s C ^s A ^s C	32
13808	0568-0587; 5' ORF	C ^s C ^s G ^s G ^s T ^s T ^s C ^s T ^s T ^s G ^s T ^s A ^s C ^s T ^s C ^s G ^s G ^s G ^s C ^s C	33
13809	0634-0653; central ORF	G ^s C ^s C ^s C ^s T ^s C ^s G ^s T ^s C ^s A ^s G ^s A ^s T ^s G ^s G ^s C ^s G ^s C ^s A	51

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13810	0829-0848; central ORF	C ^s C ^s A ^s C ^s C ^s A ^s G ^s G ^s A ^s G ^s A ^s G ^s G ^s T ^s G ^s A ^s G ^s G ^s C	34
13811	1102-1121; 3' ORF	G ^s G ^s C ^s A ^s A ^s A ^s G ^s C ^s A ^s G ^s T ^s A ^s G ^s G ^s T ^s C ^s A ^s G ^s G ^s C	35
13812	1254-1273; 3'-UTR	G ^s C ^s C ^s I ^s C ^s A ^s T ^s G ^s A ^s T ^s C ^s C ^s C ^s C ^s A ^s C ^s G ^s A ^s T ^s C	36
13872	(scrambled # 13812)	A ^s G ^s T ^s C ^s C ^s T ^s A ^s C ^s T ^s A ^s C ^s A ^s C ^s A ^s G ^s C ^s C ^s G ^s C ^s C ^s T	52
12361	0056-0075; 5' UTR	T ^s C ^s A ^s G ^s G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s A ^s C ^s T ^s T ^s C	38
12348	0056-0075; 5' UTR	T C A G G G T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s C A C T T C (2' ME)	38
12473	0056-0075; 5' UTR	T ^s C ^s A ^s G ^s G ^s G ^s T ^s A ^s A ^s G ^s A ^s C ^s T ^s C ^s A ^s C ^s T ^s T ^s C (2' F1)	38
12362	0143-0162; 5' UTR	A ^s G ^s G ^s C ^s T ^s G ^s T ^s T ^s C ^s C ^s T ^s G ^s G ^s G ^s T ^s C ^s T ^s C ^s A	39
12349	0143-0162; 5' UTR	A G G G T G ^s T ^s T ^s C ^s C ^s I ^s G ^s G ^s G ^s T C T C C A (2' ME)	39
12474	0143-0162; 5' UTR	A ^s G ^s G ^s G ^s T ^s G ^s I ^s T ^s C ^s C ^s I ^s G ^s G ^s G ^s T ^s C ^s T ^s C ^s C A (2' F1)	39
12363	0315-0334; tIR	C ^s T ^s C ^s C ^s G ^s T ^s G ^s I ^s T ^s G ^s T ^s G ^s C ^s C ^s C ^s A ^s I ^s G ^s G ^s C	40
12350	0315-0334; tIR	C T C C G T ^s G ^s I ^s T ^s G ^s T ^s G ^s C ^s C C A T G G C (2' ME)	40
12475	0315-0334; tIR	C ^s T ^s C ^s C ^s G ^s T ^s G ^s T ^s G ^s T ^s G ^s C ^s C ^s C ^s A ^s T ^s G ^s G ^s C (2' F1)	40
12364	0334-0353; 5' ORF	G ^s G ^s A ^s I ^s G ^s G ^s T ^s G ^s A ^s T ^s G ^s T ^s T ^s C ^s C ^s C ^s T ^s G ^s C	41

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12351	0334-0353; 5' ORF	G G A T G G T ^s G A T ^s G T ^s T ^s C C C T G C C (2'ME)	41
12476	0334-0353; 5' ORF	G ^s G A T ^s G G T ^s G A T ^s G T ^s T ^s C C ^s C T ^s G C C (2'F1)	41
12365	0387-0406; 5' ORF	T ^s G A ^s G A ^s A ^s G A ^s C C ^s C A ^s G C ^s C A ^s G C ^s A ^s C	42
12352	0387-0406; 5' ORF	T G A A A ^s A ^s G A ^s C C ^s A ^s G C ^s C A G C A C (2'ME)	42
12477	0387-0406; 5' ORF	T ^s G A ^s G A ^s A ^s A ^s G A ^s C C ^s A ^s G C ^s C A ^s G C ^s A ^s C (2'F1)	42
12366	0621-0640; central ORF	G ^s G C ^s C ^s G C ^s A ^s G A ^s C C ^s A ^s G G A ^s T ^s C ^s A ^s C	43
12353	0621-0640; central ORF	G G G C G C A ^s G A ^s G C C ^s A ^s G G A T C A C (2'ME)	43
12478	0621-0640; central ORF	G ^s G G C ^s C ^s G C ^s A ^s G A ^s C C ^s A ^s G ^s A ^s T ^s C ^s A ^s C (2'F1)	43
12367	1042-1061; 3' ORF	G ^s G C ^s C ^s C ^s A ^s G G A ^s T ^s G G ^s A ^s G C ^s A ^s G ^s G ^s T	44
12354	1042-1061; 3' ORF	G G C C C A ^s G G A ^s T ^s G G ^s A ^s G C A G G T (2'ME)	44
12479	1042-1061; 3' ORF	G ^s G C ^s C ^s C ^s A ^s G G A ^s T ^s G G ^s A ^s G C ^s A ^s G G ^s T (2'F1)	44
12368	1069-1088; tTR	A ^s G ^s G G C ^s G T ^s A ^s C A ^s C T ^s T ^s C C C ^s T ^s C	45
12355	1069-1088; tTR	A G G G C G T ^s A ^s C A ^s C T ^s T ^s T C C C T T C (2'ME)	45

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12480	1069-1088; tTR	A ^s G ^s G ^s G ^s C ^s G ^s T ^s A ^s C ^s A ^s C ^s T ^s T ^s T ^s C ^s C ^s C ^s T ^s T ^s C ^s	45
12369	1100-1209; tTR	C ^s A ^s G ^s C ^s C ^s C ^s C ^s T ^s T ^s T ^s G ^s C ^s T ^s T ^s C ^s T ^s G ^s C ^s G ^s G ^s A	46
12356	1100-1209; tTR	C A G C C C ^s C ^s T ^s T ^s G ^s C ^s T ^s T ^s C ^s T ^s T ^s G C G G A	46
12481	1100-1209; tTR	C ^s A ^s G ^s C ^s C ^s C ^s T ^s T ^s G ^s C ^s T ^s T ^s C ^s T ^s T ^s G ^s C ^s G ^s G ^s A	46
12370	1360-1380; 3' UTR	A ^s A ^s G ^s G ^s A ^s G ^s A ^s G ^s G ^s A ^s T ^s G ^s C ^s A ^s G ^s C ^s C ^s A	47
12357	1360-1380; 3' UTR	A A G G A G ^s A ^s G ^s G ^s G ^s A ^s T ^s G ^s C C A G C C A	47
12482	1360-1380; 3' UTR	A ^s A ^s G ^s G ^s A ^s G ^s A ^s G ^s G ^s A ^s T ^s G ^s C ^s C ^s A ^s G ^s C ^s C ^s A	47
12914	(-0038 to -0059; 5' UTR of alternative mRNA)	C ^s T ^s G ^s T ^s T ^s A ^s C ^s T ^s T ^s T ^s A ^s C ^s A ^s G ^s A ^s G ^s G ^s T ^s T ^s T ^s G	48
12915	(-0035 to -0059; 5' UTR of alternative mRNA)	C ^s T ^s T ^s C ^s T ^s G ^s T ^s T ^s A ^s C ^s T ^s T ^s T ^s A ^s C ^s A ^s G ^s A ^s G ^s G ^s G ^s T ^s T ^s T ^s G	49
13498	(-0038 to -0058; 5' UTR of alternative mRNA)	C ^s T ^s G ^s T ^s T ^s A ^s C ^s T ^s T ^s T ^s A ^s C ^s A ^s G ^s A ^s G ^s G ^s T ^s T ^s T ^s	50

TABLE 2

Sequences of Oligonucleotides Targeted to Murine B7-1 mRNA

ISIS #	Target Position; Site	Oligonucleotide Sequence (5'->3') and Chemical Modifications	SEQ ID NO:
14419	0009-0028; 5' UTR	A ^s G ^s T ^s A ^s A ^s G ^s A ^s G ^s T ^s C ^s T ^s A ^s T ^s T ^s G ^s A ^s G ^s G ^s T ^s A	53
14420	0041-0060; 5' UTR	G ^s C ^s T ^s T ^s G ^s A ^s G ^s T ^s T ^s T ^s C ^s A ^s C ^s A ^s A ^s C ^s C ^s T ^s G ^s A	54
14421	0071-0091; 5' UTR	G ^s T ^s C ^s C ^s A ^s C ^s A ^s C ^s A ^s G ^s A ^s T ^s G ^s G ^s A ^s A ^s C ^s A ^s G ^s A ^s G	55
14422	0109-0128; 5' UTR	G ^s G ^s C ^s A ^s T ^s C ^s C ^s A ^s C ^s C ^s C ^s G ^s G ^s C ^s A ^s G ^s A ^s T ^s G ^s C	56
14423	0114-0133; 5' UTR	T ^s G ^s G ^s A ^s T ^s G ^s G ^s C ^s A ^s T ^s C ^s A ^s C ^s C ^s G ^s C ^s A	57
14424	0168-0187; 5' UTR	A ^s G ^s G ^s C ^s A ^s C ^s C ^s T ^s C ^s C ^s T ^s A ^s G ^s G ^s C ^s T ^s C ^s A ^s C ^s A	58
14425	0181-0200; 5' UTR	G ^s C ^s C ^s A ^s A ^s T ^s G ^s G ^s A ^s G ^s C ^s T ^s A ^s G ^s G ^s C ^s A ^s C ^s C	59
14426	0208-0217; 5' UTR	C ^s A ^s T ^s G ^s A ^s T ^s G ^s G ^s G ^s A ^s A ^s A ^s G ^s C ^s C ^s A ^s G ^s G ^s A	60
14427	0242-0261; tIR	A ^s A ^s T ^s T ^s G ^s C ^s A ^s A ^s G ^s C ^s C ^s A ^s T ^s A ^s G ^s C ^s T ^s C ^s A	61
14428	0393-0412; 5' ORF	C ^s G ^s G ^s C ^s A ^s A ^s G ^s G ^s C ^s A ^s G ^s C ^s A ^s T ^s A ^s C ^s T ^s T	62
14909	0478-0497; 5' ORF	C ^s C ^s C ^s A ^s G ^s C ^s A ^s A ^s T ^s G ^s A ^s C ^s A ^s G ^s A ^s C ^s A ^s G ^s C ^s A	63
14910	0569-0588; central ORF	G ^s G ^s T ^s C ^s T ^s G ^s A ^s A ^s A ^s G ^s G ^s A ^s C ^s C ^s A ^s G ^s G ^s C ^s C	64
14911	0745-0764; central ORF	T ^s G ^s G ^s G ^s A ^s A ^s C ^s C ^s C ^s C ^s G ^s G ^s A ^s A ^s G ^s C ^s A	65
14912	0750-0769; central ORF	G ^s G ^s C ^s T ^s T ^s T ^s G ^s G ^s A ^s A ^s C ^s C ^s C ^s C ^s G ^s G ^s A	66

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14913	0825-0844; 3' ORF	T ^s C ^s A ^s G ^s A ^s T ^s T ^s C ^s A ^s G ^s G ^s A ^s T ^s C ^s T ^s G ^s G ^s A	67
14914	0932-0951; 3' ORF	C ^s C ^s C ^s A ^s G ^s G ^s T ^s G ^s A ^s G ^s T ^s C ^s T ^s C ^s T ^s G ^s A ^s C	68
14915	1001-1020; 3' ORF	C ^s T ^s G ^s C ^s G ^s C ^s C ^s G ^s A ^s T ^s C ^s I ^s G ^s C ^s C ^s C ^s A	69
14916	1125-1144; tTR	C ^s A ^s G ^s G ^s C ^s C ^s G ^s A ^s G ^s G ^s T ^s A ^s G ^s G ^s C ^s T ^s G	70
14917	1229-1248; 3' UTR	T ^s C ^s A ^s G ^s C ^s T ^s A ^s G ^s C ^s A ^s C ^s G ^s G ^s T ^s G ^s C ^s T ^s G ^s A ^s A	71
14918	1329-1348; 3' UTR	G ^s G ^s C ^s C ^s A ^s G ^s C ^s A ^s A ^s C ^s T ^s T ^s G ^s C ^s C ^s G ^s T	72
14919	1377-1393; 3' UTR	C ^s C ^s A ^s C ^s C ^s A ^s C ^s A ^s G ^s T ^s G ^s G ^s C ^s T ^s C ^s A ^s G ^s C ^s C	73
12912	-0067 to -0049; 5' UTR	G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s C ^s A ^s A ^s T ^s C ^s T ^s A ^s A (2' MO)	74
12913	-0067 to -0047; 5' UTR	G ^s T ^s G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s G ^s C ^s A ^s A ^s T ^s C ^s T ^s A ^s A (2' MO)	75
13496	-0067 to -0047; 5' UTR	G ^s T ^s G ^s G ^s C ^s C ^s A ^s T ^s G ^s A ^s G ^s G ^s G ^s C ^s A ^s A ^s T ^s C ^s T ^s A ^s A (2' ME)	75
13497	-0067 to -0047; 5' UTR	G T G G C C A T G A G G G C A A T C T A A (2' ME)	75

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TABLE 3

Sequences of Oligonucleotides Targeted to Human B7-2 mRNA

ISIS #	Target Position*; Site**	Oligonucleotide Sequence (5'→3')	SEQ ID NO:
9133	1367-1386; 3'-UTR	T ^s T ^s C ^s C ^s A ^s G ^s G ^s T ^s C ^s A ^s T ^s G ^s A ^s G ^s C ^s C ^s A ^s T ^s A	3
10715	scrambled control of # 9133	G ^s A ^s T ^s T ^s T ^s A ^s A ^s C ^s A ^s T ^s T ^s T ^s G ^s G ^s C ^s G ^s C ^s C ^s A	76
9134	1333-1352; 3'-UTR	C ^s A ^s T ^s A ^s A ^s G ^s G ^s T ^s G ^s T ^s G ^s C ^s T ^s C ^s T ^s G ^s A ^s A ^s G ^s T ^s G	4
9135	1211-1230; 3'-UTR	T ^s T ^s A ^s C ^s T ^s C ^s A ^s T ^s G ^s C ^s T ^s A ^s A ^s T ^s G ^s T ^s C ^s T ^s T ^s T ^s	5
9136	1101-1120; tTR	A ^s T ^s T ^s A ^s A ^s A ^s A ^s A ^s C ^s A ^s T ^s G ^s T ^s A ^s T ^s C ^s A ^s C ^s T ^s T ^s	6
10716	(scrambled # 9136)	A ^s A ^s A ^s G ^s T ^s T ^s A ^s C ^s A ^s A ^s C ^s A ^s T ^s T ^s A ^s T ^s A ^s T ^s C ^s T	77
9137	0054-0074; 5'-UTR	G ^s G ^s A ^s A ^s C ^s A ^s C ^s A ^s G ^s A ^s A ^s G ^s C ^s A ^s A ^s G ^s T ^s G ^s T ^s G ^s T	7
9138	0001-0020; 5'-UTR	C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C ^s T ^s A ^s G ^s G ^s C ^s T ^s C ^s C ^s T	8
9139	0133-0152; tIR	C ^s C ^s C ^s A ^s T ^s A ^s G ^s T ^s G ^s C ^s T ^s G ^s T ^s C ^s A ^s C ^s A ^s A ^s A ^s T	9
10877	(scrambled # 9139)	A ^s G ^s T ^s G ^s C ^s G ^s A ^s T ^s T ^s C ^s T ^s C ^s A ^s A ^s C ^s C ^s T ^s A ^s C	78
10367	0073-0092; 5'-UTR	G ^s C ^s A ^s C ^s A ^s G ^s C ^s A ^s G ^s C ^s A ^s T ^s T ^s C ^s C ^s C ^s A ^s A ^s G ^s G	10
10368	0240-0259; 5' ORF	T ^s T ^s G ^s C ^s A ^s A ^s A ^s T ^s T ^s G ^s G ^s C ^s A ^s T ^s G ^s G ^s C ^s A ^s G ^s G	11
10369	1122-1141; 3'-UTR	T ^s G ^s G ^s T ^s A ^s T ^s G ^s G ^s C ^s T ^s T ^s A ^s C ^s T ^s C ^s T ^s T	12
10370	1171-1190; 3'-UTR	A ^s A ^s A ^s G ^s G ^s T ^s T ^s G ^s C ^s C ^s A ^s G ^s G ^s A ^s C ^s G ^s G	13

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10371	1233-1252; 3'-UTR	G ^s G ^s A ^s G ^s T ^s C ^s C ^s T ^s G ^s A ^s G ^s C ^s C ^s C ^s T ^s T	14
10372	1353-1372; 3'-UTR	C ^s C ^s A ^s T ^s T ^s A ^s A ^s G ^s C ^s T ^s G ^s C ^s T ^s T ^s G ^s C ^s C	15
11149	0019-0034; 5'-UTR	T ^s A ^s T ^s T ^s T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C	79
11151	0020-0034; 5'-UTR	T ^s A ^s T ^s T ^s T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C	80
11150	0021-0034; 5'-UTR	T ^s A ^s T ^s T ^s T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C	81
10373	0011-0030; 5'-UTR	T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C	16
10721	(scrambled # 10373)	C ^s G ^s A ^s C ^s A ^s G ^s C ^s T ^s C ^s C ^s T ^s G ^s C ^s G ^s T ^s C ^s C ^s T ^s C	82
10729	(5'FITC # 10373)	T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C	16
10782	(5'cholesterol # 10373)	T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C	16
# 10373 Deletion Derivatives:			
10373	0011-0030; 5'-UTR	T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C	16
10888	0011-0026; 5'-UTR	A ^s G ^s C ^s T ^s C ^s C ^s C ^s C ^s G ^s T ^s A ^s C ^s C ^s T ^s C ^s C	83
10889	0015-0030; 5'-UTR	T ^s G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	84
10991	0015-0024; 5'-UTR	C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	85
10992	0015-0025; 5'-UTR	G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	86
10993	0015-0026; 5'-UTR	A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	87
10994	0015-0027; 5'-UTR	G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	88
10995	0015-0028; 5'-UTR	C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	89

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10996	0015-0029; 5'-UTR	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	90
11232	0017-0029; 5' UTR	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T	91
	# 10996 Derivatives:		
10996	0015-0029; 5'-UTR	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	90
11806	(scrambled # 10996)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T	92
11539	(fully 2' MO # 10996)	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90
11540	(control for # 11539)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T (2' MO)	92
11541	(# 10996 7-base "gapmer")	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90
11542	(control for # 11541)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T (2' MO)	92
11543	(# 10996 9-base "gapmer")	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90
11544	(control for # 11543)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T (2' MO)	92
11545	(# 10996 5' "wingmer")	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90
11546	(control for # 11545)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T (2' MO)	92
11547	(# 10996 3' "wingmer")	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90
11548	(control for # 11547)	G ^s C ^s C ^s G ^s C ^s C ^s G ^s C ^s A ^s A ^s G ^s T ^s C ^s T (2' MO)	92
12496	((2'-5')A ₄ # 10996)	G C G A G C T C C C G T A C	90
13107	((2'-5')A ₄ # 10996)	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C	90
12492	((2'-5')A ₄ # 10996)	G ^s C ^s G ^s A ^s G ^s C ^s T ^s C ^s C ^s C ^s G ^s T ^s A ^s C (2' MO)	90

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12495	((2'-5')A ₄ # 10996)	G ^S C ^S G ^S A ^S G ^S C ^S T ^S C ^S C ^S C ^S G ^S T ^S A ^S C (2' MO)	90
12887	(1-24 of SEQ ID NO: 1 of WO 95/03408; alternative mRNA)	G ^S A ^S G ^S A ^S A ^S G ^S C ^S A ^S A ^S A ^S G ^S C ^S T ^S T ^S T ^S C ^S A ^S C ^S C ^S C ^S -T ^S G ^S T ^S G (2' MO)	93
12888	(1-22 of SEQ ID NO: 1 of WO 95/03408; alternative mRNA)	G ^S A ^S A ^S G ^S C ^S A ^S A ^S A ^S G ^S C ^S T ^S T ^S T ^S C ^S A ^S C ^S C ^S T ^S G ^S T ^S G (2' MO)	94
12889	(1-19 of SEQ ID NO: 1 of WO 95/03408; alternative mRNA)	G ^S C ^S A ^S A ^S A ^S G ^S C ^S T ^S T ^S T ^S C ^S A ^S C ^S C ^S T ^S G ^S T ^S G (2' MO)	95
12890	0001-0024	C ^S T ^S C ^S C ^S C ^S C ^S G ^S T ^S A ^S C ^S C ^S T ^S C ^S T ^S A ^S A ^S G ^S G ^S C ^S -T ^S C ^S C ^S T (2' MO)	96
12891	0001-0022	C ^S C ^S C ^S C ^S G ^S T ^S A ^S C ^S C ^S T ^S C ^S C ^S T ^S A ^S A ^S G ^S G ^S C ^S T ^S C ^S C ^S T (2' MO)	97
12892	0001-0020	C ^S C ^S G ^S T ^S A ^S C ^S C ^S T ^S C ^S C ^S T ^S A ^S A ^S G ^S G ^S C ^S T ^S C ^S C (2' MO)	98

cDNA clones:

A cDNA encoding the sequence for human B7-1 was isolated by using the reverse transcription/polymerase chain reaction (RT-PCR). Poly A+ RNA from Daudi cells (ATCC accession No. CCL 213) was reverse transcribed using oligo-dT primer under standard conditions. Following a 30 minute reaction at 42°C and heat inactivation, the reaction mixture (20 μ L) was brought to 100 μ L with water. A 10 μ L aliquot from the RT reaction was then amplified in a 50 μ L PCR reaction using the 5' primer,

5'-GAT-CAG-GGT-ACC-CCA-AAG-AAA-AAG-TGA-TTT-GTC-ATT-GC-3' (sense, SEQ ID NO: 20), and the 3' primer,

5'-GAT-AGC-CTC-GAG-GAT-AAT-GAA-TTG-GCT-GAC-AAG-AC-3' (antisense, SEQ ID NO: 21).

The primers included unique restriction sites for subcloning of the PCR product into the vector pcDNA-3 (Invitrogen, San Diego, CA). The 5' primer was designed to have identity with bases 1 to 26 of the published human B7-1 sequence (Freeman et al., *J. Immunol.*, 1989, 143, 2714; positions 13-38 of the primer) and includes a Kpn I restriction site (positions 7-12 of the primer) for use in cloning. The 3' primer was designed to be complementary to bases 1450 to 1471 of the published sequence for B7-1 (positions 14-35 of the primer) and includes a Xho I restriction site (positions 7-12 of the primer).

Following PCR, the reaction was extracted with phenol and precipitated using ethanol. The product was digested with the appropriate restriction enzymes and the full-length fragment purified by agarose gel and ligated into the vector pcDNA-3 (Invitrogen, San Diego, CA) prepared by digesting with the same enzymes. The resultant construct, pcB7-1, was confirmed by restriction mapping and DNA sequence analysis using standard procedures. A mouse B7-1 clone, pcmb7-1, was isolated in a similar manner by RT-PCR of RNA isolated from a murine B-lymphocyte cell line, 70Z3.

A cDNA encoding the sequence for human B7-2, position 1 to 1391, was also isolated by RT-PCR. Poly A+ RNA from Daudi cells (ATCC accession No. CCL 213) was reverse transcribed using oligo-dT primer under standard conditions. Following a 30 minute reaction at 42°C and heat inactivation, the reaction mixture (20 µL) was brought to 100 µL with water. A 10 µL aliquot from the RT reaction was then amplified in a 50 µL PCR reaction using the 5' primer,

5'-GAT-CAG-GGT-ACC-AGG-AGC-CTT-AGG-AGG-TAC-GG-3'

(sense, SEQ ID NO: 1), and the 3' primer,

5'-GAT-AGC-CTC-GAG-TTA-TTT-CCA-GGT-CAT-GAG-CCA-3'

(antisense, SEQ ID NO: 2).

The 5' primer was designed to have identity with bases 1-20 of the published B7-2 sequence (Azuma *et al.*, Nature, 1993, 366, 76 and Genbank Accession No. L25259; positions 13-32 of the primer) and includes a Kpn I site (positions 7-12 of the primer) for use in cloning. The 3' primer was designed to have complementarity to bases 1370-1391 of the published sequence for B7-2 (positions 13-33 of the primer) and includes an Xho I restriction site (positions 7-12 of the primer). Following PCR, the reaction was extracted with phenol and precipitated using ethanol. The product was digested with Xho I and Kpn I, and the full-length fragment purified by agarose gel and ligated into the vector pcDNA-3 (Invitrogen, San Diego, CA) prepared by digesting with the same enzymes. The resultant construct, pcB7-2, was confirmed by restriction mapping and DNA sequence analysis using standard procedures.

A mouse B7-2 clone, pcmb7-2, was isolated in a similar manner by RT-PCR of RNA isolated from P388D1 cells using the 5' primer,

5'-GAT-CAG-GGT-ACC-AAG-AGT-GGC-TCC-TGT-AGG-CA

(sense, SEQ ID NO: 99), and the 3' primer,

5'-GAT-AGC-CTC-GAG-GTA-GAA-TTC-CAA-TCA-GCT-GA

(antisense, SEQ ID NO: 100).

The 5' primer has identity with bases 1-20, whereas the 3' primer is complementary to bases 1096-1115, of the published murine B7-2 sequence (Chen et al., *J. Immun.*, 1994, 152, 4929). Both primers incorporate the respective 5 restriction enzyme sites found in the other 5' and 3' primers used to prepare cDNA clones. The RT-PCR product was restricted with Xho I and Kpn I and ligated into pcDNA-3 (Invitrogen, San Diego, CA).

Other cDNA clones, corresponding to mRNAs resulting from alternative splicing events, are cloned in like fashion, using primers containing the appropriate restriction sites and having identity with (5' primers), or complementarity to (3' primers), the selected B7 mRNA.

Example 2: Modulation of hB7-1 Expression by Oligonucleotides

The ability of oligonucleotides to inhibit B7-1 expression was evaluated by measuring the cell surface expression of B7-1 in transfected COS-7 cells by flow cytometry.

20 Methods:

A T-175 flask was seeded at 75% confluency with COS-7 cells (ATCC accession No. CRL 1651). The plasmid pcB7-1 was introduced into cells by standard calcium phosphate transfection. Following a 4 hour transfection, the cells were trypsinized and seeded in 12-well dishes at 80% confluency. The cells were allowed to adhere to the plastic for 1 hour and were then washed with phosphate-buffered saline (PBS). OptiMEM™ (GIBCO-BRL, Gaithersburg, MD) medium was added along with 15 µg/mL of Lipofectin™ (GIBCO-BRL, Gaithersburg, MD) and oligonucleotide at the indicated concentrations. After four additional hours, the cells were washed with phosphate buffered saline (PBS) and incubated with fresh oligonucleotide

at the same concentration in DMEM (Dulbecco et al., *Virology*, 1959, 8, 396; Smith et al., *Virology*, 1960, 12, 185) with 10% fetal calf sera (FCS).

In order to monitor the effects of oligonucleotides on cell surface expression of B7-1, treated COS-7 cells were harvested by brief trypsinization 24-48 hours after oligonucleotide treatment. The cells were washed with PBS, then resuspended in 100 μ L of staining buffer (PBS, 0.2% BSA, 0.1% azide) with 5 μ L conjugated anti-B7-1-antibody (i.e., anti-hCD80-FITC, Ancell, Bayport, MN; FITC: fluorescein isothiocyanate). The cells were stained for 30 minutes at 4°C, washed with PBS, resuspended in 300 μ L containing 0.5% paraformaldehyde. Cells were harvested and the fluorescence profiles were determined using a flow cytometer.

15 Results:

The oligonucleotides shown in Table 1 were evaluated, in COS-7 cells transiently expressing B7-1 cDNA, for their ability to inhibit B7-1 expression. The results (Figure 1) identified ISIS 13805, targeted to the translation initiation codon region, and ISIS 13812, targeted to the 3' untranslated region (UTR), as the most active oligonucleotides with greater than 50% inhibition of B7-1 expression. These oligonucleotides are thus highly preferred. ISIS 13799 (targeted to the 5' untranslated region), ISIS 13802 (targeted to the 5' untranslated region), ISIS 13806 and 13807 (both targeted to the 5' region of the ORF), and ISIS 13810 (targeted to the central portion of the ORF) demonstrated 35% to 50% inhibition of B7-1 expression. These sequences are therefore also preferred.

Oligonucleotide ISIS 13800, which showed essentially no inhibition of B7-1 expression in the flow cytometry assay, and ISIS Nos. 13805 and 13812 were then evaluated for their ability to inhibit cell surface expression of B7-1 at various

concentrations of oligonucleotide. The results of these assays are shown in Figure 2. ISIS 13812 was a superior inhibitor of B7-1 expression with an IC_{50} of approximately 150 nM. ISIS 13800, targeted to the 5' UTR, was essentially inactive.

Example 3: Modulation of hB7-2 Protein by Oligonucleotides

In an initial screen, the ability of hB7-2 oligonucleotides to inhibit B7-2 expression was evaluated by measuring the cell surface expression of B7-2 in transfected COS-7 cells by flow cytometry. The methods used were similar to those given in Example 2, with the exceptions that (1) COS-7 cells were transfected with the plasmids pbcB7-2 or BBG-58, a human ICAM-1 (CD54) expression vector (R&D Systems, Minneapolis, MN) introduced into cells by standard calcium phosphate transfection, (2) the oligonucleotides used were those described in Table 2, and (3) a conjugated anti-B7-2 antibody (i.e., anti-hCD86-FITC or anti-CD86-PE, PharMingen, San Diego, CA; PE: phycoerythrin) was used during flow cytometry.

Results:

The results are shown in Figure 3. At a concentration of 200 nM, ISIS 9133, ISIS 9139 and ISIS 10373 exhibited inhibitory activity of 50% or better and are therefore highly preferred. These oligonucleotides are targeted to the 3' untranslated region (ISIS 9133), the translation initiation codon region (ISIS 9139) and the 5' untranslated region (ISIS 10373). At the same concentration, ISIS 10715, ISIS 10716 and ISIS 10721, which are scrambled controls for ISIS 9133, ISIS 9139 and ISIS 10373, respectively, showed no inhibitory activity. Treatment with ISIS 10367 and ISIS 10369 resulted in greater than 25% inhibition, and these oligonucleotides are

thus also preferred. These oligonucleotides are targeted to the 5' (ISIS 10367) and 3' (ISIS 10369) untranslated regions.

Example 4: Modulation of *hB7-2* mRNA by Oligonucleotides

Methods:

5 For ribonuclease protection assays, cells were harvested 18 hours after completion of oligonucleotide treatment using a Totally RNA™ kit (Ambion, Austin, TX). The probes for the assay were generated from plasmids pcB7-2 (linearized by digestion with Bgl II) and pTRI-b-actin (Ambion Inc., Austin, 10 TX). In vitro transcription of the linearized plasmid from the SP6 promoter was performed in the presence of α -³²P-UTP (800 Ci/mmol) yielding an antisense RNA complementary to the 3' end of B7-2 (position 1044-1391). The probe was gel-purified after treatment with DNase I to remove DNA template.

15 Ribonuclease protection assays were carried out using an RPA II™ kit (Ambion) according to the manufacturer's directions. Total RNA (5 μ g) was hybridized overnight, at 42°C, with 10⁵ cpm of the B7-2 probe or a control beta-actin probe. The hybridization reaction was then treated, at 37°C for 30 20 minutes, with 0.4 units of RNase A and 2 units of RNase T1. Protected RNA was precipitated, resuspended in 10 μ L of gel loading buffer and electrophoresed on a 6% acrylamide gel with 50% w/v urea at 20 W. The gel was then exposed and the lanes quantitated using a PhosphorImager (Molecular Dynamics, 25 Sunnyvale, CA) essentially according to the manufacturer's instructions.

Results:

The extent of oligonucleotide-mediated *hB7-2* mRNA modulation generally paralleled the effects seen for *hB7-2* 30 protein (Table 5). As with the protein expression (flow cytometry) assays, the most active oligonucleotides were ISIS 9133, ISIS 9139 and 10373. None of the oligonucleotides

tested had an inhibitory effect on the expression of b-actin mRNA in the same cells.

TABLE 5

Activities of Oligonucleotides Targeted to hB7-2 mRNA

5	ISIS NO.	SEQ ID NO.	% Control Protein	% Control RNA Expression
	9133	3	70.2	46.0
	9134	4	88.8	94.5
	9135	5	98.2	83.4
	9136	6	97.1	103.1
10	9137	7	80.5	78.1
	9138	8	86.4	65.9
	9139	9	47.9	32.6
	10367	10	71.3	52.5
	10368	11	81.0	84.5
15	10369	12	71.3	81.5
	10370	13	84.3	83.2
	10371	14	97.3	92.9
	10372	15	101.7	82.5
	10373	16	43.5	32.7

20 **Example 5: Additional hB7-1 and hB7-2 Oligonucleotides**

Oligonucleotides having structures and/or sequences that were modified relative to the oligonucleotides identified during the initial screening were prepared. These oligonucleotides were evaluated for their ability to modulate
25 human B7-2 expression using the methods described in the previous examples.

ISIS 10996, an oligonucleotide having a 15 nucleotide sequence derived from the 20 nucleotide sequence of ISIS 10373, was also prepared and evaluated. ISIS 10996 comprises

15 nucleotides, 5'-GCG-AGC-TCC-CCG-TAC (SEQ ID NO: 90) contained within the sequence of ISIS 10373. Both ISIS 10373 and 10996 overlap a potential stem-loop structure located within the B7-2 message comprising bases 1-67 of the sequence
5 of hB7-2 presented by Azuma *et al.* (*Nature*, 1993, 366, 76). While not intending to be bound by any particular theory regarding their mode(s) of action, ISIS 10373 and ISIS 10996 have the potential to bind as loop 1 pseudo-half-knots at a secondary structure within the target RNA. U.S. Patent
10 5,5152,438, the contents of which are hereby incorporated by reference, describes methods for modulating gene expression by the formation of pseudo-half-knots. Regardless of their mode(s) of action, despite having a shorter length than ISIS 10373, the 15-mer ISIS 10996 is as (or more) active in the B7-
15 2 protein expression assay than the 20-mer from which it is derived (Figure 4; ISIS 10721 is a scrambled control for ISIS 10373). A related 16-mer, ISIS 10889, was also active in the B7-2 protein expression assay. However, a structurally related 14-mer (ISIS 10995), 13-mer (ISIS 10994), 12-mer (ISIS
20 10993), 11-mer (ISIS 10992) and 10-mer (ISIS 10991) exhibited little or no activity in this assay. ISIS 10996 was further derivatized in the following ways.

ISIS 10996 derivatives having 2' methoxethoxy substitutions were prepared, including a fully substituted
25 derivative (ISIS 11539), "gapmers" (ISIS 11541 and 11543) and "wingmers" (ISIS 11545 and 11547). As explained in Example 5, the 2' methoxyethoxy substitution prevents the action of some nucleases (e.g., RNase H) but enhances the affinity of the modified oligonucleotide for its target RNA molecule.
30 These oligonucleotides are tested for their ability to modulate hB7-2 message or function according to the methods of Examples 3, 4, 7 and 8.

ISIS 10996 derivatives were prepared in order to be evaluated for their ability to recruit RNase L to a target RNA
35 molecule, e.g., hB7-2 message. RNase L binds to, and is

activated by, (2'-5')(A)_n, which is in turn produced from ATP by (2'-5')(A)_n synthetase upon activation by, e.g., interferon. RNase L has been implicated in antiviral mechanisms and in the regulation of cell growth as well
5 (Sawai, *Chemica Scripta*, 1986, 21, 169; Charachon et al., *Biochemistry*, 1990, 29, 2550). The combination of anti-B7 oligonucleotides conjugated to (2'-5')(A)_n is expected to result in the activation of RNase L and its targeting to the B7 message complementary to the oligonucleotide sequence. The
10 following oligonucleotides have identical sequences (i.e., that of ISIS 10996) and identical (2'-5')(A)₄ "caps" on their 5' termini: ISIS 12492, 12495, 12496 and 13107. The adenosyl residues have 3' hydroxyl groups and are linked to each other by phosphorothioate linkages. The (3'-5') portion of the
15 oligonucleotide, which has a sequence complementary to a portion of the human B7-2 RNA, is conjugated to the (2'-5')(A)₄ "cap" via a phosphorothioate linkage from the 5' residue of the (3'-5') portion of the oligonucleotide to an n-aminohexyl linker which is bonded to the "cap" via
20 another phosphorothioate linkage. In order to test a variety of chemically diverse oligonucleotides of this type for their ability to recruit RNase L to a specific message, different chemical modifications were made to this set of four oligonucleotides as follows. ISIS 12496 consists of
25 unmodified oligonucleotides in the (3'-5') portion of the oligonucleotide. In ISIS 13107, phosphorothioate linkages replace the phosphate linkages found in naturally occurring nucleic acids. Phosphorothioate linkages are also employed in ISIS 12492 and 12495, which additionally have 2'-
30 methoxyethoxy substitutions. These oligonucleotides are tested for their ability to modulate hB7-2 message or function according to the methods of Examples 3, 4, 7 and 8.

Derivatives of ISIS 10996 having modifications at the 2' position were prepared and evaluated. The modified
35 oligonucleotides included ISIS 11539 (fully 2'-O-methyl), ISIS

11541 (having 2'-O-methyl "wings" and a central 7-base "gap"),
ISIS 11543 (2'-O-methyl wings with a 9-base gap), ISIS 11545
(having a 5' 2'-O-methyl wing) and ISIS 11547 (having a 3' 2'-
O-methyl wing). The results of assays of 2'-O-methyl
5 oligonucleotides were as follows. ISIS 11539, the fully 2'-O-
methyl version of ISIS 10996, was not active at all in the
protein expression assay. The gapped and winged
oligonucleotides (ISIS 11541, 11543, 11545 and 11547) each
showed some activity at 200 nM (i.e., from 60 to 70%
10 expression relative to untreated cells), but less than that
demonstrated by the parent compound, ISIS 10996 (i.e., about
50% expression). Similar results were seen in RNA expression
assays.

ISIS 10782, a derivative of ISIS 10373 to which
15 cholesterol has been conjugated via a 5' *n*-aminohexyl linker,
was prepared. Lipophilic moieties such as cholesterol have
been reported to enhance the uptake by cells of
oligonucleotides in some instances, although the extent to
which uptake is enhanced, if any, remains unpredictable. ISIS
20 10782, and other oligonucleotides comprising lipophilic
moieties, are tested for their ability to modulate B7-2
message or function according to the methods of Examples 3,
4, 7 and 8.

A series of 2'-methoxyethoxy (herein, "2'ME") and 2'-
25 fluoride (herein, "2'F") "gapmer" derivatives of the *h*B7-1
oligonucleotides ISIS 12361 (ISIS Nos. 12348 and 12473,
respectively), ISIS 12362 (ISIS Nos. 12349 and 12474), ISIS
12363 (ISIS Nos. 12350 and 12475), ISIS 12364 (ISIS Nos. 12351
and 12476), ISIS 12365 (ISIS Nos. 12352 and 12477), ISIS 12366
30 (ISIS Nos. 12353 and 12478), ISIS 12367 (ISIS Nos. 12354 and
12479), ISIS 12368 (ISIS Nos. 12355 and 12480), ISIS 12369
(ISIS Nos. 12356 and 12481) and ISIS 12370 (ISIS Nos. 12357
and 12482) were prepared. The central, non-2'-modified
portions (Agaps@) of these derivatives support RNase H
35 activity when the oligonucleotide is bound to its target RNA,

even though the 2'-modified portions do not. However, the 2'-modified "wings" of these oligonucleotides enhance their affinity to their target RNA molecules (Cook, Chapter 9 In: *Antisense Research and Applications*, Crooke et al., eds., CRC Press, Boca Raton, 1993, pp. 171-172).

Another 2' modification is the introduction of a methoxy (MO) group at this position. Like 2'ME- and 2'F-modified oligonucleotides, this modification prevents the action of RNase H on duplexes formed from such oligonucleotides and their target RNA molecules, but enhances the affinity of an oligonucleotide for its target RNA molecule. ISIS 12914 and 12915 comprise sequences complementary to the 5' untranslated region of alternative hB7-1 mRNA molecules, which arise from alternative splicing events of the primary hB7-1 transcript. These oligonucleotides include 2' methoxy modifications, and the enhanced target affinity resulting therefrom may allow for greater activity against alternatively spliced B7-1 mRNA molecules which may be present in low abundance in some tissues (Inobe et al., *J. Immun.*, 1996, 157, 582). Similarly, ISIS 13498 and 13499, which comprise antisense sequences to other alternative hB7-1 mRNAs, include 2' methoxyethoxy modifications in order to enhance their affinity for their target molecules, and 2' methoxyethoxy or 2'methoxy substitutions are incorporated into the hB7-2 oligonucleotides ISIS 12912, 12913, 13496 and 13497. These oligonucleotides are tested for their ability to modulate hB7-1 essentially according to the methods of Example 2 or hB7-2 according to the methods of Examples 3, 4, 7 and 8, with the exception that, when necessary, the target cells are transfected with a cDNA clone corresponding to the appropriate alternatively spliced B7 transcript.

Example 6: Specificity of Antisense Modulation

Several oligonucleotides of the invention were evaluated in a cell surface expression flow cytometry assay to determine

the specificity of the oligonucleotides for B7-1 as contrasted with activity against B7-2. The oligonucleotides tested in this assay included ISIS 13812, an inhibitor of B7-1 expression (Figure 1; Example 2) and ISIS 10373, an inhibitor of B7-2 expression (Figure 3; Example 3). The results of this assay are shown in Figure 5. ISIS 13812 inhibits B7-1 expression with little or no effect on B7-2 expression. As is also seen in Figure 5, ISIS 10373 inhibits B7-2 expression with little or no effect on B7-1 expression. ISIS 13872 (SEQ ID NO: 37, AGT-CCT-ACT-ACC-AGC-CGC-CT), a scrambled control of ISIS 13812, and ISIS 13809 (SEQ ID NO: 51) were included in these assays and demonstrated essentially no activity against either B7-1 or B7-2.

Example 7: Modulation of hB7-2 Expression by Oligonucleotides in Antigen Presenting Cells

The ability of ISIS 10373 to inhibit expression from the native B7-2 gene in antigen presenting cells (APCs) was evaluated as follows.

Methods:

Monocytes were cultured and treated with oligonucleotides as follows. For dendritic cells, EDTA-treated blood was layered onto Polymorphprep™ (1.113 g/mL; Nycomed, Oslo, Norway) and sedimented at 500x g for 30 minutes at 20°C. Mononuclear cells were harvested from the interface. Cells were washed with PBS, with serum-free RPMI media (Moore et al., N.Y. J. Med., 1968, 68, 2054) and then with RPMI containing 5% fetal bovine serum (FBS). Monocytes were selected by adherence to plastic cell culture cell culture dishes for 1 h at 37°C. After adherence, cells were treated with oligonucleotides in serum-free RPMI containing Lipofectin™ (8 µg/mL). After 4 hours, the cells were washed. Then RPMI containing 5% FBS and oligonucleotide was added to cells along with interleukin-4 (IL-4; R&D Systems, Minneapolis, MN) (66 ng/mL) and granulocyte-macrophage colony-

stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN) (66 ng/mL) to stimulate differentiation (Romani et al., *J. Exp. Med.*, 1994, 180, 83, 1994). Cells were incubated for 48 hours, after which cell surface expression of various
5 molecules was measured by flow cytometry.

Mononuclear cells isolated from fresh blood were treated with oligonucleotide in the presence of cationic lipid to promote cellular uptake. As a control oligonucleotide, ISIS 2302 (an inhibitor of ICAM-1 expression; SEQ ID NO: 17) was
10 also administered to the cells. Expression of B7-2 protein was measured by flow cytometry according to the methods of Example 2. Monoclonal antibodies not described in the previous Examples included anti-hCD3 (Ancell, Bayport, MN) and anti-HLA-DR (Becton Dickinson, San Jose, CA).

15 **Results:**

As shown in Figure 6, ISIS 10373 has a significant inhibitory effect on B7-2 expression with an IC_{50} of approximately 250 nM. ISIS 10373 had only a slight effect on ICAM-1 expression even at a dose of 1 μ M. ISIS 2302 (SEQ ID NO: 17), a control
20 oligonucleotide which has been shown to inhibit ICAM-1 expression, had no effect on B7-2 expression, but significantly decreased ICAM-1 levels with an IC_{50} of approximately 250 nM. Under similar conditions, ISIS 10373 did not affect the cell surface expression of B7-1, HLA-DR or
25 CD3 as measured by flow cytometry.

Example 8: Modulation of T Cell Proliferation by Oligonucleotides

The ability of ISIS 2302 and ISIS 10373 to inhibit T cell proliferation was evaluated as follows. Monocytes treated
30 with oligonucleotide and cytokines (as in Example 6) were used as antigen presenting cells in a T cell proliferation assay. The differentiated monocytes were combined with CD4+ T cells

from a separate donor. After 48 hours, proliferation was measured by [³H] thymidine incorporation.

Methods:

For T cell proliferation assays, cells were isolated from EDTA-treated whole blood as described above, except that a faster migrating band containing the lymphocytes was harvested from just below the interface. Cells were washed as described in Example 6 after which erythrocytes were removed by NH₄Cl lysis. T cells were purified using a T cell enrichment column (R&D Systems, Minneapolis, MN) essentially according to the manufacturer's directions. CD4⁺ T cells were further enriched from the entire T cell population by depletion of CD8⁺ cells with anti-CD8-conjugated magnetic beads (AMAC, Inc., Westbrook, ME) according to the manufacturer's directions. T cells were determined to be >80% CD4⁺ by flow cytometry using Cy-chrome-conjugated anti-CD4 mAb (PharMingen, San Diego, CA).

Antigen presenting cells (APCs) were isolated as described in Example 6 and treated with mitomycin C (25 µg/mL) for 1 hour then washed 3 times with PBS. APCs (10⁵ cells) were then combined with 4 x 10⁴ CD4⁺ T cells in 350 µL of culture media. Where indicated, purified CD3 mAb was also added at a concentration of 1 µg/mL. During the last 6 hours of the 48 hour incubation period, proliferation was measured by determining uptake of 1.5 uCi of [³H]-thymidine per well. The cells were harvested onto filters and the radioactivity measured by scintillation counting.

Results:

As shown in Figure 7, mononuclear cells which were not cytokine-treated slightly induced T cell proliferation, presumably due to low levels of costimulatory molecules expressed on the cells. However, when the cells were treated with cytokines and induced to differentiate to dendritic-like

cells, expression of both ICAM-1 and B7-2 was strongly upregulated. This resulted in a strong T cell proliferative response which could be blocked with either anti-ICAM-1 (ISIS 2302) or anti-B7-2 (ISIS 10373) oligonucleotides prior to
5 induction of the mononuclear cells. The control oligonucleotide (ISIS 10721) had an insignificant effect on T cell proliferation. A combination treatment with both the anti-ICAM-1 (ISIS 2302) and anti-B7-2 (ISIS 10373) oligonucleotides resulted in a further decrease in T cell
10 response.

Example 9: Modulation of Murine B7 Genes by Oligonucleotides

Oligonucleotides (see Table 4) capable of inhibiting expression of murine B7-2 transiently expressed in COS-7 cells were identified in the following manner. A series of
15 phosphorothioate oligonucleotides complementary to murine B7-2 (mB7-2) cDNA were screened for their ability to reduce mB7-2 levels (measured by flow cytometry as in Example 2, except that a conjugated anti-mB7-2 antibody (i.e., anti-mCD86-PE, PharMingen, San Diego, CA) in COS-7 cells transfected with an
20 mB7-2 cDNA clone. Anti-mB7-2 antibody may also be obtained from the hybridoma deposited at the ATCC under accession No. HB-253. Oligonucleotides (see Table 2) capable of modulating murine B7-1 expression are isolated in like fashion, except that a conjugated anti-
25 mB7-1 antibody is used in conjunction with COS-7 cells transfected with an mB7-1 cDNA clone.

For murine B7-2, the most active oligonucleotide identified was ISIS 11696 (GGA-TTG-CCA-AGC-CCA-TGG-TG, SEQ ID NO: 18), which is complementary to position 96-115 of the
30 cDNA, a site which includes the translation initiation (AUG) codon. Figure 8 shows a dose-response curve for ISIS 11696 and a scrambled control, ISIS 11866 (CTA-AGT-AGT-GCT-AGC-CGG-GA, SEQ ID NO: 19). ISIS 11696 inhibited cell surface expression of B7-2 in COS-7 cells with an IC_{50} in the range of
35 200-300 nM, while ISIS 11866 exhibited less than 20%

inhibition at the highest concentration tested (1000 nM).

In order to further evaluate the murine B7-2 antisense oligonucleotides, the IC-21 cell line was used. IC-21 monocyte/macrophage cell line expresses both B7-1 and murine
5 B7-2 (mB7-2) constitutively. A 2-fold induction of expression can be achieved by incubating the cells in the presence of lipopolysaccharide (LPS; GIBCO-BRL, Gaithersburg, MD) (Hathcock et al., *Science*, 1993, 262, 905).

IC-21 cells (ATCC; accession No. TIB 186) were seeded at 80%
10 confluency in 12-well plates in DMEM media with 10% FCS. The cells were allowed to adhere to the plate overnight. The following day, the medium was removed and the cells were washed with PBS. Then 500 μ L of OptiMEM™ (GIBCO-BRL, Gaithersburg, MD) supplemented with 15 μ g/mL of Lipofectin™
15 (GIBCO-BRL, Gaithersburg, MD) was added to each well. Oligonucleotides were then added directly to the medium at the indicated concentrations. After incubation for 4 hours, the cells were washed with PBS and incubated overnight in culture medium supplemented with 15 μ g/mL of LPS. The following day,
20 cells were harvested by scraping, then analyzed for cell surface expression by flow cytometry.

ISIS 11696 and ISIS 11866 were administered to IC-21 cells in the presence of Lipofectin™ (GIBCO-BRL, Gaithersburg, MD). The results are shown in Figure 9. At a concentration
25 of 10 μ M, ISIS 11696 inhibited mB7-2 expression completely (and decreased mB7-2 levels below the constitutive level of expression), while the scrambled control oligonucleotide, ISIS 11866, produced only a 40% reduction in the level of induced expression. At a concentration of 3 μ M, levels of induced
30 expression were greatly reduced by ISIS 11696, while ISIS 11866 had little effect.

Modified oligonucleotides, comprising 2' substitutions (e.g., 2' methoxy, 2' methoxyethoxy) and targeted to alternative transcripts of murine B7-1 (ISIS 12914, 12915,

13498, 13499) or murine B7-2 (ISIS 13100, 13100 and 13102) were prepared. These oligonucleotides are tested for their ability to modulate murine B7 essentially according to the above methods using IC-21 cells or COS-7 transfected with a
5 cDNA clone corresponding to the appropriate alternatively spliced B7 transcript.

Example 10: Modulation of Allograft Rejection by Oligonucleotides

A murine model for evaluating compounds for their ability
10 to inhibit heart allograft rejection has been previously described (Stepkowski et al., *J. Immunol.*, 1994, 153, 5336). This model was used to evaluate the immunosuppressive capacity of antisense oligonucleotides to B7 proteins alone or in combination with antisense oligonucleotides to intercellular
15 adhesion molecule-1 (ICAM-1).

Methods:

Heart allograft rejection studies and oligonucleotide treatments of BALB/c mice were performed essentially as previously described (Stepkowski et al., *J. Immunol.*, 1994,
20 153, 5336). Antisense oligonucleotides used included ISIS 11696, ISIS 3082 (targeted to ICAM-1) and ISIS 1082 (a control oligonucleotide targeted to the herpes virus UL-13 gene sequence). Dosages used were 1, 2, 2.5, 5 or 10 mg/kg of individual oligonucleotide (as indicated below); when
25 combinations of oligonucleotides were administered, each oligonucleotide was given at a dosage of 1, 5 or 10 mg/kg (total oligonucleotide dosages of 2, 10 and 20 mg/kg, respectively). The survival times of the transplanted hearts and their hosts were monitored and recorded.

30 Results:

The mean survival time for untreated mice was 8.2 ± 0.8 days (7,8,8,8,9,9 days). Treatment of the mice for 7 days with

ISIS 1082 (SEQ ID NO: 125, unrelated control oligonucleotide) slightly reduced the mean survival times to 7.1 ± 0.7 days (5 mg/kg/day; 6,7,7,7,8,8) or 7.0 ± 0.8 days (10 mg/kg/day; 6,7,7,8). Treatment of the mice for seven days with the murine B7-2 oligonucleotide ISIS 11696 (SEQ ID NO: 108) increased the mean survival time to 9.3 days at two doses (2 mg/kg/day, 9.3 ± 0.6 days, 9,9,10; 10 mg/kg/day, 9.3 ± 1.3 days, 8,9,9,11). Treatment of mice for seven days with an ICAM-1 oligonucleotide, ISIS 3082, also increased the mean survival of the mice over several doses. Specifically, at 1 mg/kg/day, the mean survival time (MSD) was 11.0 ± 0.0 (11,11,11); at 2.5 mg/kg/day, the MSD was 12.0 ± 2.7 (10,12,13,16); at 5 mg/kg/day, the MSD was 14.1 ± 2.7 (10,12,12,13,16,16,17,17); and, at 10 mg/kg/day, the MSD was 15.3 ± 5.8 (12,12,13,24). Some synergistic effect was seen when the mice were treated for seven days with 1 mg/kg/day each of ISIS 3082 and 11696: the MSD was 13.8 ± 1.0 (13,13,14,15).

Example 11: Detection of Nucleic Acids Encoding B7 Proteins

Oligonucleotides are radiolabeled after synthesis by ^{32}P -labeling at the 5' end with polynucleotide kinase. Sambrook et al., "Molecular Cloning. A Laboratory Manual," Cold Spring Harbor Laboratory Press, 1989, Volume 2, pg. 11.31. Radiolabeled oligonucleotide capable of hybridizing to a nucleic acid encoding a B7 protein is contacted with a tissue or cell sample suspected of B7 protein expression under conditions in which specific hybridization can occur, and the sample is washed to remove unbound oligonucleotide. A similar control is maintained wherein the radiolabeled oligonucleotide is contacted with a normal tissue or cell sample under conditions that allow specific hybridization, and the sample is washed to remove unbound oligonucleotide. Radioactivity remaining in the samples indicates bound oligonucleotide and is quantitated using a scintillation counter or other routine

means. A greater amount of radioactivity remaining in the samples, as compared to control tissues or cells, indicates increased expression of a B7 gene, whereas a lesser amount of radioactivity in the samples relative to the controls
5 indicates decreased expression of a B7 gene.

Radiolabeled oligonucleotides of the invention are also useful in autoradiography. A section of tissues suspected of expressing a B7 gene is treated with radiolabeled oligonucleotide and washed as described above, then exposed
10 to photographic emulsion according to standard autoradiography procedures. A control of a normal tissue section is also maintained. The emulsion, when developed, yields an image of silver grains over the regions expressing a B7 gene, which is quantitated. The extent of B7 expression is determined by
15 comparison of the silver grains observed with control and test samples.

Analogous assays for fluorescent detection of expression of a B7 gene use oligonucleotides of the invention which are labeled with fluorescein or other fluorescent tags. Labeled
20 oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems, Foster City, CA) using standard phosphoramidite chemistry. b-Cyanoethyldiisopropyl phosphoramidites are purchased from Applied Biosystems (Foster City, CA). Fluorescein-labeled amidites are purchased from
25 Glen Research (Sterling, VA). Incubation of oligonucleotide and biological sample is carried out as described above for radiolabeled oligonucleotides except that, instead of a scintillation counter, a fluorescence microscope is used to detect the fluorescence. A greater amount of fluorescence in
30 the samples, as compared to control tissues or cells, indicates increased expression of a B7 gene, whereas a lesser amount of fluorescence in the samples relative to the controls indicates decreased expression of a B7 gene.

Example 12: Chimeric (deoxy gapped) Human B7-1 Antisense Oligonucleotides

Additional oligonucleotides targeting human B7-1 were synthesized. Oligonucleotides were synthesized as uniformly 5 phosphorothioate chimeric oligonucleotides having regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 6.

Oligonucleotides were screened as described in Example 10 4. Results are shown in Table 7.

Oligonucleotides 22315 (SEQ ID NO: 128), 22316 (SEQ ID NO: 26), 22317 (SEQ ID NO: 129), 22320 (SEQ ID NO: 132), 22324 (SEQ ID NO: 135), 22325 (SEQ ID NO: 136), 22334 (SEQ ID NO: 145), 22335 (SEQ ID NO: 146), 22337 (SEQ ID NO: 148), and 15 22338 (SEQ ID NO: 36) resulted in 50% or greater inhibition of B7-1 mRNA in this assay.

TABLE 6:

Nucleotide Sequences of Human B7-1 Chimeric (deoxy gapped) Oligodeoxynucleotides

20	ISIS	NUCLEOTIDE SEQUENCE ¹	SEQ	TARGET GENE	GENE
	NO.	(5' -> 3')	ID	NUCLEOTIDE	TARGET
			NO:	CO-ORDINATES ²	REGION
	22313	AGACTCCACTTCTGAGATGT	126	0048-0067	5'-UTR
	22314	TGAAGAAAAATTCCACTTTT	127	0094-0113	5'-UTR
	22315	TTTAGTTTCACAGCTTGCTG	128	0112-0129	5'-UTR
25	22316	GCTCACGTAGAAGACCCTCC	26	0193-0212	5'-UTR
	22317	TCCCAGGTGCAAAACAGGCA	129	0233-0252	5'-UTR
	22318	GTGAAAGCCAACAATTTGGA	130	0274-0293	5'-UTR
	22319	CATGGCTTCAGATGCTTAGG	131	0301-0320	AUG
	22320	TTGAGGTATGGACACTTGGA	132	0351-0370	coding
30	22321	GACCAGCCAGCACCAAGAGC	31	0380-0399	coding
	22322	GCGTTGCCACTTCTTTCACT	133	0440-0459	coding

5	22323	TTTTGCCAGTAGATGCGAGT	134	0501-0520	coding
	22324	GGCCATATATTCATGTCCCC	135	0552-0571	coding
	22325	GCCAGGATCACAATGGAGAG	136	0612-0631	coding
	22326	GTATGTGCCCTCGTCAGATG	137	0640-0659	coding
	22327	TTCAGCCAGGTGTTCCCGCT	138	0697-0716	coding
10	22328	GGAAGTCAGCTTTGACTGAT	139	0725-0744	coding
	22329	CCTCCAGAGGTTGAGCAAAT	140	0798-0817	coding
	22330	CCAACCAGGAGAGGTGAGGC	141	0827-0846	coding
	22331	GAAGCTGTGGTTGGTTGTCA	142	0940-0959	coding
	22332	TTGAAGGTCTGATTCACTCT	143	0987-1006	coding
15	22333	AAGGTAATGGCCCAGGATGG	144	1050-1069	coding
	22334	AAGCAGTAGGTCAGGCAGCA	145	1098-1117	coding
	22335	CCTTGCTTCTGCGGACACTG	146	1185-1204	3'-UTR
	22336	AGCCCCCTTGCTTCTGCGGAC	147	1189-1208	3'-UTR
	22337	TGACGGAGGCTACCTTCAGA	148	1216-1235	3'-UTR
20	22338	GCCTCATGATCCCCACGATC	36	1254-1273	3'-UTR
	22339	GTAAACAGCTTAAATTTGT	149	1286-1305	3'-UTR
	22340	AGAAGAGGTTACATTAAGCA	150	1398-1417	3'-UTR
	22341	AGATAATGAATTGGCTGACA	151	1454-1473	3'-UTR
	24733	GCGTCATCATCCGCACCATC	152	control	
	24734	CGTTGCTTGTGCCGACAGTG	153	control	
	24735	GCTCACGAAGAACACCTTCC	154	control	

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. M27533, locus name "HUMIGB7".

TABLE 7

Inhibition of Human B7-1 mRNA Expression by Chimeric (deoxy
gapped) Phosphorothioate Oligodeoxynucleotides

	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
5	basal	---	---	100%	---
	13805	30	AUG	46%	54%
	13812	36	3'-UTR	22%	78%
	22313	126	5'-UTR	75%	25%
10	22314	127	5'-UTR	69%	31%
	22315	128	5'-UTR	49%	51%
	22316	26	5'-UTR	42%	58%
	22317	129	5'-UTR	43%	57%
	22318	130	5'-UTR	63%	37%
15	22319	131	AUG	68%	32%
	22320	132	coding	45%	55%
	22321	31	coding	57%	43%
	22324	135	coding	46%	54%
	22325	136	coding	46%	54%
20	22326	137	coding	62%	38%
	22328	139	coding	64%	36%
	22329	140	coding	59%	41%
	22330	141	coding	54%	46%
	22331	142	coding	62%	38%
25	22332	143	coding	67%	33%
	22333	144	coding	73%	27%
	22334	145	coding	43%	57%
	22335	146	3'-UTR	43%	57%
	22336	147	3'-UTR	55%	45%
30	22337	148	3'-UTR	42%	58%
	22338	36	3'-UTR	40%	60%
	22339	149	3'-UTR	69%	31%

22340	150	3'-UTR	71%	29%
22341	151	3'-UTR	59%	41%

Dose response experiments were performed on several of the more active oligonucleotides. The oligonucleotides were
5 screened as described in Example 4 except that the concentration of oligonucleotide was varied as shown in Table 8. Mismatch control oligonucleotides were included. Results are shown in Table 8.

All antisense oligonucleotides tested showed a dose
10 response effect with inhibition of mRNA approximately 60% or greater.

TABLE 8

Dose Response of COS-7 Cells to B7-1

Chimeric (deoxy gapped) Antisense Oligonucleotides

ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% mRNA Expression	% mRNA Inhibition
basal	---	---	---	100%	---
22316	26	5'-UTR	10 nM	99%	1%
"	"	"	30 nM	73%	27%
"	"	"	100 nM	58%	42%
"	"	"	300 nM	33%	67%
24735	154	control	10 nM	100%	---
"	"	"	30 nM	95%	5%
"	"	"	100 nM	81%	19%
"	"	"	300 nM	75%	25%
22335	146	3'-UTR	10 nM	81%	19%
"	"	"	30 nM	63%	37%
"	"	"	100 nM	43%	57%
"	"	"	300 nM	35%	65%
24734	153	control	10 nM	94%	6%
"	"	"	30 nM	96%	4%
"	"	"	100 nM	94%	6%

5	"	"	"	300 nM	84%	16%
	22338	36	3'-UTR	10 nM	68%	32%
	"	"	"	30 nM	60%	40%
	"	"	"	100 nM	53%	47%
	"	"	"	300 nM	41%	59%
	24733	152	control	10 nM	90%	10%
	"	"	"	30 nM	91%	9%
	"	"	"	100 nM	90%	10%
	"	"	"	300 nM	80%	20%
	"	"	"	300 nM	80%	20%

10 Example 13: Chimeric (deoxy gapped) Mouse B7-1 Antisense Oligonucleotides

Additional oligonucleotides targeting mouse B7-1 were synthesized. Oligonucleotides were synthesized as uniformly phosphorothioate chimeric oligonucleotides having 15 regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 9.

Oligonucleotides were screened as described in Example 4. Results are shown in Table 10. Oligonucleotides 18105 (SEQ ID NO: 156), 18106 (SEQ ID NO: 157), 18109 (SEQ ID NO: 160), 18110 (SEQ ID NO: 161), 18111 (SEQ ID NO: 162), 18112 (SEQ ID NO: 163), 18113 (SEQ ID NO: 164), 18114 (SEQ ID NO: 165), 18115 (SEQ ID NO: 166), 18117 (SEQ ID NO: 168), 18118 (SEQ ID NO: 169), 18119 (SEQ ID NO: 170), 18120 (SEQ ID NO: 171), 18122 (SEQ ID NO: 173), and 18123 (SEQ ID NO: 174) resulted in greater than approximately 50% inhibition of B7-1 mRNA in this assay.

TABLE 9

Nucleotide Sequences of Mouse B7-1 Chimeric (deoxy gapped)
Oligodeoxynucleotides

5	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
	18104	AGAGAACTAGTAAGAGTCT	155	0018-0037	5'-UTR
	18105	TGGCATCCACCCGGCAGATG	156	0110-0129	5'-UTR
	18106	TCGAGAAACAGAGATGTAGA	157	0144-0163	5'-UTR
	18107	TGGAGCTTAGGCACCTCCTA	158	0176-0195	5'-UTR
10	18108	TGGGGAAAGCCAGGAATCTA	159	0203-0222	5'-UTR
	18109	CAGCACAAAGAGAAGAATGA	160	0310-0329	coding
	18110	ATGAGGAGAGTTGTAAACGGC	161	0409-0428	coding
	18111	AAGTCCGGTTCTTATACTCG	162	0515-0534	coding
	18112	GCAGGTAATCCTTTTAGTGT	163	0724-0743	coding
15	18113	GTGAAGTCCTCTGACACGTG	164	0927-0946	coding
	18114	CGAATCCTGCCCCAAAGAGC	165	0995-1014	coding
	18115	ACTGCGCCGAATCCTGCCCC	166	1002-1021	coding
	18116	TTGATGATGACAACGATGAC	167	1035-1054	coding
	18117	CTGTTGTTTGTCTCTGCT	168	1098-1117	coding
20	18118	TGTTCACTAATGCTTCTTC	169	1134-1153	coding
	18119	GTAACTCTATCTTGTGTCA	170	1263-1282	3'-UTR
	18120	TCCACTTCAGTCATCAAGCA	171	1355-1374	3'-UTR
	18121	TGCTCAATACTCTCTTTT	172	1680-1699	3'-UTR
	18122	AGGCCAGCAAACCTTGCCCG	173	1330-1349	3'-UTR
25	18123	AACGGCAAGGCAGCAATACC	174	0395-0414	coding

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

30 ²Co-ordinates from Genbank Accession No. X60958, locus name "MMB7BLAA".

TABLE 10

Inhibition of Mouse B7-1 mRNA Expression by Chimeric (deoxy gapped) Phosphorothioate Oligodeoxynucleotides

5	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
	basal	---	---	100.0%	---
	18104	155	5'-UTR	60.0%	40.0%
	18105	156	5'-UTR	32.0%	68.0%
	18106	157	5'-UTR	51.0%	49.0%
10	18107	158	5'-UTR	58.0%	42.0%
	18108	159	5'-UTR	82.0%	18.0%
	18109	160	coding	45.5%	54.5%
	18110	161	coding	21.0%	79.0%
	18111	162	coding	38.0%	62.0%
15	18112	163	coding	42.0%	58.0%
	18113	164	coding	24.6%	75.4%
	18114	165	coding	25.6%	74.4%
	18115	166	coding	33.5%	66.5%
	18116	167	coding	65.6%	34.4%
20	18117	168	coding	46.7%	53.3%
	18118	169	coding	31.7%	68.3%
	18119	170	3'-UTR	24.0%	76.0%
	18120	171	3'-UTR	26.7%	73.3%
	18121	172	3'-UTR	114.0%	---
25	18122	173	3'-UTR	42.0%	58.0%
	18123	174	coding	42.0%	58.0%

Example 14: Chimeric (deoxy gapped) Human B7-2 Antisense Oligonucleotides

Additional oligonucleotides targeting human B7-2 were
 30 synthesized. Oligonucleotides were synthesized as uniformly
 phosphorothioate chimeric oligonucleotides having regions of

five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 11.

Oligonucleotides were screened as described in Example 4. Results are shown in Table 12. Oligonucleotides 22284 (SEQ ID NO: 16), 22286 (SEQ ID NO: 176), 22287 (SEQ ID NO: 177), 22288 (SEQ ID NO: 178), 22289 (SEQ ID NO: 179), 22290 (SEQ ID NO: 180), 22291 (SEQ ID NO: 181), 22292 (SEQ ID NO: 182), 22293 (SEQ ID NO: 183), 22294 (SEQ ID NO: 184), 22296 (SEQ ID NO: 186), 22299 (SEQ ID NO: 189), 22300 (SEQ ID NO: 190), 22301 (SEQ ID NO: 191), 22302 (SEQ ID NO: 192), 22303 (SEQ ID NO: 193), 22304 (SEQ ID NO: 194), 22306 (SEQ ID NO: 196), 22307 (SEQ ID NO: 197), 22308 (SEQ ID NO: 198), 22309 (SEQ ID NO: 199), 22310 (SEQ ID NO: 200), and 22311 (SEQ ID NO: 201) resulted in greater than 50% inhibition of B7-2 mRNA in this assay.

TABLE 11

**Nucleotide Sequences of Human B7-2 Chimeric (deoxy gapped)
Oligodeoxynucleotides**

ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
22284	TGCGAGCTCCCCGTACCTCC	16	0011-0030	5'-UTR
22285	CAGAAGCAAGGTGGTAAGAA	175	0049-0068	5'-UTR
22286	GCCTGTCCACTGTAGCTCCA	176	0113-0132	5'-UTR
22287	AGAATGTTACTCAGTCCCAT	177	0148-0167	AUG
22288	TCAGAGGAGCAGCACCAGAG	178	0189-0208	coding
22289	TGGCATGGCAGGTCTGCAGT	179	0232-0251	coding
22290	AGTCACTCAGGCTTTGGTT	180	0268-0287	coding
22291	TGCCTAAGTATACCTCATTC	181	0324-0343	coding
22292	CTGTCAAATTTCTCTTTGCC	182	0340-0359	coding

	22293	CATATACTT GGAATGAACAC	183	0359-0378	coding
	22294	GGTCCA ACTGTCCGAATCAA	184	0392-0411	coding
	22295	TGATCT GAAAGATTGTGAAGT	185	0417-0436	coding
	22296	AAGCCCTT GTCTTGATCTG	186	0430-0449	coding
5	22297	TGTGAT GGATGATACATTGA	187	0453-0472	coding
	22298	TCAGGT TGACTGAAGTTAGC	188	0529-0548	coding
	22299	GTGTAT AGATGAGCAGGTCA	189	0593-0612	coding
	22300	TCTGTG ACATTATCTTGAGA	190	0694-0713	coding
	22301	AAGATAAA AGCCGCGTCTTG	191	0798-0817	coding
10	22302	AGAAAACC ATCACACATATA	192	0900-0919	coding
	22303	AGAGTT GCGAGGCCGCTTCT	193	0947-0968	coding
	22304	TCCCTCT CCATTGTGTTGGT	194	0979-0998	coding
	22305	CATCAG ATCTTTCAGGTATA	195	1035-1054	coding
	22306	GGCTTT ACTCTTTAATTAAA	196	1115-1134	stop
15	22307	GAAATCA AAAAGGTTGCCCA	197	1178-1197	3'-UTR
	22308	GGAGTC CTGGAGCCCCCTTA	198	1231-1250	3'-UTR
	22309	TTGGCA TACGGAGCAGAGCT	199	1281-1300	3'-UTR
	22310	TGTGCT CTGAAGTGAAAAGA	200	1327-1346	3'-UTR
	22311	GGCTTG GCCCATAGTGTGC	201	1342-1361	3'-UTR
20	22312	CCTAAAT TTTATTTCCAGGT	202	1379-1398	3'-UTR
	24736	GCTCCA AGTGTCCCAATGAA	203	control	
	24737	AGTATG TTTCTCACTCCGAT	204	control	
	24738	TGCCAG CACCCGGTACGTCC	205	control	

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. U04343 locus name "HSU04343".

TABLE 12

Inhibition of Human B7-2 mRNA Expression by Chimeric (deoxy
gapped) Phosphorothioate Oligodeoxynucleotides

5	ISIS	SEQ ID	GENE	% mRNA	% mRNA
	No:	NO:	TARGET REGION	EXPRESSION	INHIBITION
	basal	---	---	100%	0%
	10373	16	5'-UTR	24%	76%
	22284	16	5'-UTR	30%	70%
	22285	175	5'-UTR	74%	26%
10	22286	176	5'-UTR	39%	61%
	22287	177	AUG	27%	73%
	22288	178	coding	38%	62%
	22289	179	coding	41%	59%
	22290	180	coding	42%	58%
15	22291	181	coding	41%	59%
	22292	182	coding	39%	61%
	22293	183	coding	43%	57%
	22294	184	coding	21%	79%
	22295	185	coding	66%	34%
20	22296	186	coding	42%	58%
	22297	187	coding	54%	46%
	22298	188	coding	53%	47%
	22299	189	coding	46%	54%
	22300	190	coding	39%	61%
25	22301	191	coding	51%	49%
	22302	192	coding	41%	59%
	22303	193	coding	46%	54%
	22304	194	coding	41%	59%
	22305	195	coding	57%	43%
30	22306	196	stop	44%	56%
	22307	197	3'-UTR	45%	55%
	22308	198	3'-UTR	40%	60%

22309	199	3'-UTR	42%	58%
22310	200	3'-UTR	41%	59%
22311	201	3'-UTR	49%	51%
22312	202	3'-UTR	83%	17%

5 Dose response experiments were performed on several of the more active oligonucleotides. The oligonucleotides were screened as described in Example 4 except that the concentration of oligonucleotide was varied as shown in Table 13. Mismatch control oligonucleotides were included. Results
10 are shown in Table 13.

All antisense oligonucleotides tested showed a dose response effect with maximum inhibition of mRNA approximately 50% or greater.

TABLE 13

15 Dose Response of COS-7 Cells to B7-2
Chimeric (deoxy gapped) Antisense Oligonucleotides

	SEQ ID	ASO Gene		% mRNA	% mRNA
ISIS #	NO:	Target	Dose	Expression	Inhibition
basal	---	---	---	100%	---
22284	16	5'-UTR	10 nM	92%	8%
20 "	"	"	30 nM	72%	28%
"	"	"	100 nM	59%	41%
"	"	"	300 nM	48%	52%
24738	205	control	10 nM	81%	19%
"	"	"	30 nM	92%	8%
25 "	"	"	100 nM	101%	---
"	"	"	300 nM	124%	---
22287	177	AUG	10 nM	93%	7%
"	"	"	30 nM	79%	21%
"	"	"	100 nM	66%	34%
30 "	"	"	300 nM	45%	55%
24737	204	control	10 nM	85%	15%

5	"	"	"	30 nM	95%	5%
	"	"	"	100 nM	87%	13%
	"	"	"	300 nM	99%	1%
	22294	184	coding	10 nM	93%	7%
	"	"	"	30 nM	95%	5%
10	"	"	"	100 nM	58%	42%
	"	"	"	300 nM	45%	55%
	24736	203	control	10 nM	102%	---
	"	"	"	30 nM	101%	---
	"	"	"	100 nM	100%	---
	"	"	"	300 nM	107%	---

Example 15: Chimeric (deoxy gapped) Mouse B7-2 Antisense Oligonucleotides

Additional oligonucleotides targeting mouse B7-2 were synthesized. Oligonucleotides were synthesized as uniformly phosphorothioate chimeric oligonucleotides having regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 14.

Oligonucleotides were screened as described in Example 4. Results are shown in Table 15.

Oligonucleotides 18084 (SEQ ID NO: 206), 18085 (SEQ ID NO: 207), 18086 (SEQ ID NO: 208), 18087 (SEQ ID NO: 209), 18089 (SEQ ID NO: 211), 18090 (SEQ ID NO: 212), 18091 (SEQ ID NO: 213), 18093 (SEQ ID NO: 215), 18095 (SEQ ID NO: 217), 18096 (SEQ ID NO: 218), 18097 (SEQ ID NO: 219), 18098 (SEQ ID NO: 108), 18102 (SEQ ID NO: 223), and 18103 (SEQ ID NO: 224) resulted in 50% or greater inhibition of B7-2 mRNA expression in this assay.

TABLE 14
Nucleotide Sequences of Mouse B7-2 Chimeric (deoxy gapped)
Oligodeoxynucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
18084	GCTGCCTACAGGAGCCACTC	206	0003-0022	5'-UTR
18085	TCAAGTCCGTGCTGCCTACA	207	0013-0032	5'-UTR
18086	GTCTACAGGAGTCTGGTTGT	208	0033-0052	5'-UTR
18087	AGCTTGCGTCTCCACGGAAA	209	0152-0171	coding
18088	TCACACTATCAAGTTTCTCT	210	0297-0316	coding
18089	GTCAAAGCTCGTGCGGCCCA	211	0329-0348	coding
18090	GTGAAGTCGTAGAGTCCAGT	212	0356-0375	coding
18091	GTGACCTTGCTTAGACGTGC	213	0551-0570	coding
18092	CATCTTCTTAGGTTTCGGGT	214	0569-0588	coding
18093	GGCTGTTGGAGATACTGAAC	215	0663-0682	coding
18094	GGGAATGAAAGAGAGAGGCT	216	0679-0698	coding
18095	ACATACAATGATGAGCAGCA	217	0854-0873	coding
18096	GTCTCTCTGTCAGCGTTACT	218	0934-0953	coding
18097	TGCCAAGCCCATGGTGCATC	219	0092-0111	AUG
18098	GGATTGCCAAGCCCATGGTG	108	0096-0115	AUG
18099	GCAATTTGGGGTTCAAGTTC	220	0967-0986	coding
18100	CAATCAGCTGAGAACATTTT	221	1087-1106	3'-UTR
18101	TTTTGTATAAAACAATCATA	222	0403-0422	coding
18102	CCTTCACTCTGCATTTGGTT	223	0995-1014	stop
18103	TGCATGTTATCACCATACTC	224	0616-0635	coding

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. S70108 locus name "S70108".

TABLE 15

Inhibition of Mouse B7-2 mRNA Expression by Chimeric (deoxy
gapped) Phosphorothioate Oligodeoxynucleotides

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ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
basal	---	---	100.0%	0.0%
18084	206	5'-UTR	36.4%	63.6%
18085	207	5'-UTR	35.0%	65.0%
18086	208	5'-UTR	40.1%	59.9%
18087	209	coding	42.1%	57.9%
18088	210	coding	52.3%	47.7%
18089	211	coding	20.9%	79.1%
18090	212	coding	36.6%	63.4%
18091	213	coding	37.1%	62.9%
18092	214	coding	58.9%	41.1%
18093	215	coding	32.7%	67.3%
18094	216	coding	63.8%	36.2%
18095	217	coding	34.3%	65.7%
18096	218	coding	32.3%	67.7%
18097	219	AUG	24.5%	75.5%
18098	108	AUG	32.2%	67.8%
18099	220	coding	66.8%	33.2%
18100	221	3'-UTR	67.2%	32.8%
18101	222	coding	88.9%	11.1%
18102	223	stop	33.8%	66.2%
18103	224	coding	30.2%	69.8%

Example 16: Effect of B7 Antisense Oligonucleotides on Cell

30 Surface Expression

B7 antisense oligonucleotides were tested for their

effect on cell surface expression of both B7-1 and B7-2. Cell surface expression was measured as described in Example 2. Experiments were done for both human B7 and mouse B7. Results for human B7 are shown in Table 16.

5 Results for mouse B7 are shown in Table 17.

In both species, B7-1 antisense oligonucleotides were able to specifically reduce the cell surface expression of B7-1. B7-2 antisense oligonucleotides were specific for the B7-2 family member. These oligonucleotides were also specific
10 for their effect on B7-1 and B7-2 mRNA levels.

TABLE 16

**Inhibition of Human B7 Cell Surface Expression by Chimeric
(deoxy gapped) Phosphorothioate Oligodeoxynucleotides**

15	ISIS No:	SEQ ID NO:	GENE TARGET	% B7-1 EXPRESSION	% B7-2 EXPRESSION
	basal	---	---	100%	0%
	22316	26	B7-1	31%	100%
	22317	129	B7-1	28%	91%
	22320	132	B7-1	37%	86%
20	22324	135	B7-1	37%	91%
	22325	136	B7-1	32%	89%
	22334	145	B7-1	28%	92%
	22335	146	B7-1	23%	95%
	22337	148	B7-1	48%	101%
25	22338	36	B7-1	22%	96%
	22284	16	B7-2	88%	32%
	22287	177	B7-2	92%	35%
	22294	184	B7-2	77%	28%

TABLE 17

Inhibition of Mouse B7 Cell Surface Expression by Chimeric
(deoxy gapped) Phosphorothioate Oligodeoxynucleotides

5	ISIS	SEQ	GENE	% B7-1	% B7-2
	No:	ID	TARGET	EXPRESSION	EXPRESSION
		NO:	REGION		
	basal	---	---	100%	0%
	18089	211	B7-2	85%	36%
	18097	219	B7-2	87%	28%
	18110	161	B7-1	31%	93%
10	18113	164	B7-1	25%	91%
	18119	170	B7-1	27%	98%

Dose response experiments were performed on several of the more active human B7-1 antisense oligonucleotides. The oligonucleotides were screened as described in Example 2 except that the concentration of oligonucleotide was varied as shown in Table 18. Results are shown in Table 18.

All antisense oligonucleotides tested showed a dose response effect with inhibition of cell surface expression approximately 60% or greater.

TABLE 18

Dose Response of COS-7 Cells to B7-1
Chimeric (deoxy gapped) Antisense Oligonucleotides

25	ISIS #	SEQ ID	ASO Gene		% Surface	% Surface
		NO:	Target	Dose	Expression	Inhibition
	basal	---	---	---	100%	---
	22316	26	5'-UTR	10 nM	74%	26%
	"	"	"	30 nM	74%	26%
	"	"	"	100 nM	47%	53%
	"	"	"	300 nM	34%	66%
	22335	146	3'-UTR	10 nM	81%	19%

5	"	"	"	30 nM	69%	31%
	"	"	"	100 nM	47%	53%
	"	"	"	300 nM	38%	62%
	22338	36	3'-UTR	10 nM	78%	22%
	"	"	"	30 nM	65%	35%
	"	"	"	100 nM	50%	50%
	"	"	"	300 nM	40%	60%

Dose response experiments were performed on several of the more active human B7-2 antisense oligonucleotides. The oligonucleotides were screened as described in Example 2 except that the concentration of oligonucleotide was varied as shown in Table 19. Results are shown in Table 19.

All antisense oligonucleotides tested showed a dose response effect with maximum inhibition of cell surface expression 85% or greater.

TABLE 19

Dose Response of COS-7 Cells to B7-2

Chimeric (deoxy gapped) Antisense Oligonucleotides

	ISIS #	SEQ ID NO:	ASO Gene Target	Dose	% Surface Expression	% Surface Inhibition
20	basal	---	---	---	100%	---
	22284	16	5'-UTR	10 nM	63%	37%
	"	"	"	30 nM	60%	40%
	"	"	"	100 nM	37%	63%
	"	"	"	300 nM	15%	85%
25	22287	177	AUG	10 nM	93%	7%
	"	"	"	30 nM	60%	40%
	"	"	"	100 nM	32%	68%
	"	"	"	300 nM	15%	85%
	22294	184	coding	10 nM	89%	11%
30	"	"	"	30 nM	62%	38%
	"	"	"	100 nM	29%	71%
	"	"	"	300 nM	12%	88%

EXAMPLE 17: Effect of B7-1 Antisense Oligonucleotides in a Murine Model for Rheumatoid Arthritis

Collagen-induced arthritis (CIA) was used as a murine model for arthritis (Mussener, A., et al., Clin. Exp. Immunol., 5 1997, 107, 485-493). Female DBA/1LacJ mice (Jackson Laboratories, Bar Harbor, ME) between the ages of 6 and 8 weeks were used to assess the activity of B7-1 antisense oligonucleotides.

On day 0, the mice were immunized at the base of the tail 10 with 100 μ g of bovine type II collagen which is emulsified in Complete Freund's Adjuvant (CFA). On day 7, a second booster dose of collagen was administered by the same route. On day 14, the mice were injected subcutaneously with 100 μ g of LPS. Oligonucleotide was administered intraperitoneally daily (10 15 mg/kg bolus) starting on day -3 (three days before day 0) and continuing for the duration of the study. Oligonucleotide 17456 (SEQ ID NO. 173) is a fully phosphorothioated analog of 18122.

Weights were recorded weekly. Mice were inspected daily 20 for the onset of CIA. Paw widths and rear ankle widths of affected and unaffected joints were measured three times a week using a constant tension caliper. Limbs were clinically evaluated and graded on a scale from 0-4 (with 4 being the highest).

Results are shown in Table 20. Treatment with B7-1 and 25 B7-2 antisense oligonucleotides was able to reduce the incidence of the disease, but had modest effects on severity. The combination of 17456 (SEQ ID NO. 173) and 11696 (SEQ ID NO. 108) was able to significantly reduce the incidence of the 30 disease and its severity.

TABLE 20

Effect of B7 antisense oligonucleotide on CIA

ISIS #(s)	SEQ ID NO	Dose mg/kg	% Incidence	Peak day ¹	Severity ²
control		---	70%	6.7 ± 2.9	3.2 ± 1.1
5 17456 (B7-1)	173	10	50%	12.1 ± 4.6	2.7 ± 1.3
11696 (B7-2)	108	10	37.5%	11.6 ± 4.5	3.4 ± 1.8
10 17456/11696		10	30%	1.0 ± 0.6	0.7 ± 0.4
18110 (B7-1)	161	10	55.6%	2.0 ± 0.8	2.0 ± 1.3
18089 (B7-2)	211	10	44.4%	6.8 ± 2.2	2.3 ± 1.3
18110/18089		10	60%	11.6 ± 0.7	4.5 ± 1.7

15 ¹Peak day is the day from onset of maximum swelling for each joint measure.

²Severity is the total clinical score divided by the total number of mice in the group.

20 EXAMPLE 18: Effect of B7-1 Antisense Oligonucleotides in a Murine Model for Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is a commonly accepted murine model for multiple sclerosis (Myers, K.J., et al., J. Neuroimmunol., 1992, 41, 1-8). SJL/H, PL/J, (SJLxPL/J)F1, (SJLxBalb/c)F1 and Balb/c female mice 25 between the ages of 6 and 12 weeks are used to test the activity of a B7-1 antisense oligonucleotide.

The mice are immunized in the two rear foot pads and base of the tail with an emulsion consisting of encephalitogenic protein or peptide (according to Myers, K.J., et al., J. of 30 Immunol., 1993, 151, 2252-2260) in Complete Freund's Adjuvant

supplemented with heat killed Mycobacterium tuberculosis. Two days later, the mice receive an intravenous injection of 500 ng Bordatella pertussis toxin and additional adjuvant.

Alternatively, the disease may also be induced by the adoptive transfer of T-cells. T-cells are obtained from the draining of the lymph nodes of mice immunized with encephalitogenic protein or peptide in CFA. The T cells are grown in tissue culture for several days and then injected intravenously into naive syngeneic recipients.

Mice are monitored and scored daily on a 0-5 scale for signals of the disease, including loss of tail muscle tone, wobbly gait, and various degrees of paralysis.

Oligonucleotide 17456 (SEQ ID NO. 173), a fully phosphorothioated analog of 18122, was compared to a saline control and a fully phosphorothioated oligonucleotide of random sequence (Oligonucleotide 17460). Results of this experiment are shown in Figure 11.

As shown in Figure 11, for all doses of oligonucleotide 17456 tested, there is a protective effect, i.e. a reduction of disease severity. At 0.2 mg/kg, this protective effect is greatly reduced after day 20, but at the higher doses tested, the protective effect remains throughout the course of the experiment (day 40). The control oligonucleotide gave results similar to that obtained with the saline control.

EXAMPLE 19: Additional antisense oligonucleotides targeted to human B7-1

Additional oligonucleotides targeting human B7-1 were synthesized. Oligonucleotides were synthesized as uniformly phosphorothioate chimeric oligonucleotides having regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides. Oligonucleotide sequences are shown in Table 21.

The human promonocytic leukaemia cell line, THP-1 (American Type Culture Collection, Manassas, VA) was maintained in RPMI 1640 growth media supplemented with 10% fetal calf serum (FCS; Life Technologies, Rockville, MD). A total of 1×10^7 cells were electroporated at an oligonucleotide concentration of 10 micromolar in 2 mm cuvettes, using an Electrocell Manipulator 600 instrument (Biotechnologies and Experimental Research, Inc.) employing 200 V, 1000 μ F. Electroporated cells were then transferred to petri dishes and allowed to recover for 16 hrs. Cells were then induced with LPS at a final concentration of 1 μ g/ml for 16 hours. RNA was isolated and processed as described in previous examples. Results are shown in Table 22.

Oligonucleotides 113492, 113495, 113498, 113499, 113501, 113502, 113504, 113505, 113507, 113510, 113511, 113513 and 113514 (SEQ ID NO: 228, 231, 234, 235, 237, 238, 240, 241, 243, 246, 247, 249 and 250) resulted in 50% or greater inhibition of B7-1 mRNA expression in this assay.

TABLE 21

Nucleotide Sequences of Human B7-1 Chimeric (deoxy gapped)
Oligodeoxynucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO.	TARGET GENE NUCLEOTIDE CO-ORDINATES ²	GENE TARGET REGION
113489	CCCTCCAGTGATGTTTACAA	225	179	5' UTR
113490	GAAGACCCCTCCAGTGATGTT	226	184	5' UTR
113491	CGTAGAAGACCCCTCCAGTGA	227	188	5' UTR
113492	TTCCCAGGTGCAAAACAGGC	228	234	5' UTR
113493	TGGCTTCAGATGCTTAGGGT	229	299	5' UTR
113494	CCTCCGTGTGTGGCCCATGG	230	316	AUG
113495	GGTGATGTTCCCTGCCTCCG	231	330	Coding
113496	GATGGTGATGTTCCCTGCCT	232	333	Coding

	113497	AGGTATGGACACTTGGATGG	233	348	Coding
	113498	GAAAGACCAGCCAGCACCAA	234	384	Coding
	113499	CAGCGTTGCCACTTCTTTCA	235	442	Coding
	113500	GTGACCACAGGACAGCGTTG	236	454	Coding
5	113501	AGATGCGAGTTTGTGCCAGC	237	491	Coding
	113502	CCTTTTGCCAGTAGATGCGA	238	503	Coding
	113503	CGGTTCTTGTACTCGGGCCA	239	567	Coding
	113504	CGCAGAGCCAGGATCACAAT	240	618	Coding
	113505	CTTCAGCCAGGTGTTCCCGC	241	698	Coding
10	113506	TAACGTCACTTCAGCCAGGT	242	706	Coding
	113507	TTCTCCATTTTCCAACCAGG	243	838	Coding
	113508	CTGTTGTGTTGATGGCATT	244	863	Coding
	113509	CATGAAGCTGTGGTTGGTTG	245	943	Coding
	113510	AGGAAAATGCTCTTGCTTGG	246	1018	Coding
15	113511	TGGGAGCAGGTTATCAGGAA	247	1033	Coding
	113512	TAAGGTAATGGCCAGGATG	248	1051	Coding
	113513	GGTCAGGCAGCATATCACAA	249	1090	Coding
	113514	GCCCCTTGCTTCTGCGGACA	250	1188	3' UTR
	113515	AGATCTTTTCAGCCCCTTGC	251	1199	3' UTR
20	113516	TTTGTTAAGGGAAGAATGCC	252	1271	3' UTR
	113517	AAAGGAGAGGGATGCCAGCC	253	1362	3' UTR
	113518	CAAGACAATTCAAGATGGCA	254	1436	3' UTR

¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy
 25 cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

²Co-ordinates from Genbank Accession No. M27533 to which the oligonucleotides are targeted.

TABLE 22

Inhibition of Human B7-1 mRNA Expression by Chimeric (deoxy
gapped) Phosphorothioate Oligodeoxynucleotides

	ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
5	113489	225	5' UTR	122	--
	113490	226	5' UTR	183	--
	113491	227	5' UTR	179	--
	113492	228	5' UTR	27	73
10	113493	229	5' UTR	488	--
	113494	230	AUG	77	23
	113495	231	Coding	43	57
	113496	232	Coding	71	29
	113497	233	Coding	78	22
15	113498	234	Coding	37	63
	113499	235	Coding	25	75
	113500	236	Coding	83	17
	113501	237	Coding	36	64
	113502	238	Coding	26	74
20	113503	239	Coding	65	35
	113504	240	Coding	46	54
	113505	241	Coding	40	60
	113506	242	Coding	105	--
	113507	243	Coding	36	64
25	113508	244	Coding	117	--
	113509	245	Coding	62	38
	113510	246	Coding	43	57
	113511	247	Coding	48	52
	113512	248	Coding	73	27
30	113513	249	Coding	48	52
	113514	250	3' UTR	35	65
	113515	251	3' UTR	184	--
	113516	252	3' UTR	83	17

113517	253	3' UTR	201	--
113518	254	3' UTR	97	03

EXAMPLE 20: Additional antisense oligonucleotides targeted to human B7-2

5 Additional oligonucleotides targeting human B7-2 were synthesized. Oligonucleotides were synthesized as uniformly phosphorothioate chimeric oligonucleotides having regions of five 2'-O-methoxyethyl (2'-MOE) nucleotides at the wings and a central region of ten deoxynucleotides.

10 Oligonucleotide sequences are shown in Table 23.

The human promonocytic leukaemia cell line, THP-1 (American Type Culture Collection, Manassas, VA) was maintained in RPMI 1640 growth media supplemented with 10% fetal calf serum (FCS; Life Technologies, Rockville, MD). A

15 total of 1×10^7 cells were electroporated at an oligonucleotide concentration of 10 micromolar in 2 mm cuvettes, using an Electrocell Manipulator 600 instrument (Biotechnologies and Experimental Research, Inc.) employing 200 V, 1000 μ F. Electroporated cells were then transferred

20 to petri dishes and allowed to recover for 16 hrs Cells were then induced with LPS and dibutyryl cAMP (500 μ M) for 16 hours. RNA was isolated and processed as described in previous examples. Results are shown in Table 24.

Oligonucleotides ISIS 113131, 113132, 113134, 113138,

25 113142, 113144, 113145, 113146, 113147, 113148, 113149, 113150, 113153, 113155, 113157, 113158, 113159 and 113160 (SEQ ID NO: 255, 256, 258, 262, 266, 268, 269, 270, 271, 272, 273, 274, 277, 279, 281, 282, 283 and 284) resulted in 50% or greater inhibition of B7-2 mRNA expression in this assay.

TABLE 23:

Nucleotide Sequences of Human B7-2 Chimeric (deoxy gapped)
Oligodeoxynucleotides

5	ISIS NO.	NUCLEOTIDE SEQUENCE ¹ (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE CO- ORDINATES ²	GENE TARGET REGION
	113131	CGTGTGTCTGTGCTAGTCCC	255	38	5' UTR
	113132	GCTGCTTCTGCTGTGACCTA	256	83	5' UTR
	113133	TATTTGCGAGCTCCCCGTAC	257	15	5' UTR
	113134	GCATAAGCACAGCAGCATTTC	258	79	5' UTR
10	113135	TCCAAAAAGAGACCAGATGC	259	97	5' UTR
	113136	AAATGCCTGTCCACTGTAGC	260	117	5' UTR
	113137	CTTCAGAGGAGCAGCACCAG	261	191	Coding
	113138	GAATCTTCAGAGGAGCAGCA	262	195	Coding
	113139	CAAATTGGCATGGCAGGTCT	263	237	Coding
	113140	GCTTTGGTTTTGAGAGTTTG	264	257	Coding
	113141	AGGCTTTGGTTTTGAGAGTT	265	259	Coding
	113142	GCTCACTCAGGCTTTGGTTT	266	267	Coding
15	113143	GGTCCTGCCAAAATACTACT	267	288	Coding
	113144	AGCCCTTGTCCTTGATCTGA	268	429	Coding
	113145	TGTGGGCTTTTGTGATGGA	269	464	Coding
	113146	AATCATTCCTGTGGGCTTTT	270	473	Coding
20	113147	CCGTGTATAGATGAGCAGGT	271	595	Coding
	113148	ACCGTGTATAGATGAGCAGG	272	596	Coding
	113149	TCATCTTCTTAGGTTCTGGG	273	618	Coding
	113150	ACAAGCTGATGGAAACGTCG	274	720	Coding
25	113151	TGCTCGTAACATCAGGGAAT	275	747	Coding
	113152	AAGATGGTCATATTGCTCGT	276	760	Coding
	113153	CGCGTCTTGTCAGTTTCCAG	277	787	Coding
	113154	CAGCTGTAATCCAAGGAATG	278	864	Coding
30	113155	GGGCTTCATCAGATCTTTCA	279	1041	Coding
	113156	CATGTATCACTTTTGTGCGCA	280	1093	Coding

113157	AGCCCCCTTATTACTCATGG	281	1221	3' UTR
113158	GGAGTTACAGGGAGGCTATT	282	1261	3' UTR
113159	AGTCTCCTCTTGGCATAACGG	283	1290	3' UTR
113160	CCCATAAGTGTGCTCTGAAG	284	1335	3' UTR

5 ¹ Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethyl cytosines and 2'-deoxy cytosines residues are 5-methyl-cytosines; all linkages are phosphorothioate linkages.

²For ISIS# 113131 and 113132, co-ordinates are from Genbank
10 Accession No. L25259, locus name "HUMB72A". For remaining
oigonucleotides, co-ordinates are from Genbank Accession
No.U04343, locus name "HSU04343".

TABLE 24

Inhibition of Human B7-2 mRNA Expression by Chimeric (deoxy
15 gapped) Phosphorothioate Oligodeoxynucleotides

ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
113131	255	5' UTR	13	87
113132	256	5' UTR	17	83
20 113133	257	5' UTR	214	--
113134	258	5' UTR	27	73
113135	259	5' UTR	66	34
113136	260	5' UTR	81	19
113137	261	Coding	57	43
25 113138	262	Coding	12	88
113140	264	Coding	214	--
113141	265	Coding	126	--
113142	266	Coding	35	65
113143	267	Coding	118	--

5	113144	268	Coding	41	59
	113145	269	Coding	46	54
	113146	270	Coding	32	68
	113147	271	Coding	35	65
	113148	272	Coding	23	77
10	113149	273	Coding	29	71
	113150	274	Coding	19	81
	113151	275	Coding	208	--
	113152	276	Coding	89	11
	113153	277	Coding	19	81
15	113154	278	Coding	63	37
	113155	279	Coding	13	87
	113156	280	Coding	83	17
	113157	281	3' UTR	13	87
	113158	282	3' UTR	20	80
	113159	283	3' UTR	43	57
	113160	284	3' UTR	09	91

EXAMPLE 21: Human skin psoriasis model

20 Animal models of psoriasis based on xenotransplantation of human skin from psoriatic patients are advantageous because they involve the direct study of affected human tissue. Psoriasis is solely a disease of the skin and consequently, engraftment of human psoriatic skin onto SCID mice allows
 25 psoriasis to be created with a high degree of fidelity in mice.

BALB/cByJSmn-Prkdcscid/J SCID mice (4-6 weeks old) of either sex (Jackson Laboratory, Bar Harbor, ME) were maintained in a pathogen free environment. At 6-8 weeks of
 30 age, mice were anesthetized by intraperitoneal injection of 30 mg/kg body weight ketamine-HCl and 1 mg/kg body weight acepromazine. After anesthesia, mice were prepared for transplantation by shaving the hair from the dorsal skin, 2

cm away from the head. The area was then sterilized and cleaned with povidone iodide and alcohol. Graft beds of about 1 cm x 1 cm were created on the shaved areas by removing full thickness skin down to the fascia. Partial thickness human skin was then orthotopically transferred onto the graft bed. The transplants were held in place by gluing the human skin to mouse-to-mouse skin with Nexband liquid, a veterinary bandage (Veterinary Products Laboratories, Phoenix, AZ). Finally, the transplant and the wounds were covered with a thick layer of antibiotic ointment. After 4 weeks of transplantation, a 2 mm punch biopsy was obtained to confirm the acceptance of the graft and the origin of the skin in the transplant area. Only mice whose grafts did not show signs of infection were used for the study. Normal human skin was obtained from elective plastic surgeries and psoriatic plaques were obtained from shave biopsies from psoriatic volunteers. Partial thickness skin was prepared by dermatome shaving of the skin and transplanted to the mouse as described above for the psoriatic skin.

Animals (n=5) were topically treated with 2.5% (w/w) of each antisense oligonucleotide in a cream formulation comprising 10% isopropyl myristate, 10% glyceryl monooleate, 3% cetostearyl alcohol, 10% polyoxyl-20-cetyl ether, 6% poloxamer 407, 2.5% phenoxyethanol, 0.5% methylparaben, 0.5% propylparaben and water (final pH about 7.5).

The following oligonucleotides were used: human B7-1 (5'-TTCCCAGGTGCAAAACAGGC-3'; SEQ ID NO: 228) (ISIS 113492) and human B7-2 (5'-CGTGTGTCTGTGCTAGTCCC-3'; SEQ ID NO: 255) (ISIS 113131). Both sequences contained only phosphorothioate linkages and had 2'-MOE modifications at nucleotides 1-5 and 16-20.

Plaques from the same patients were also transplanted onto control mice (n=5) and treated only with the vehicle of the active cream preparation. Both groups received the topical preparation twice a day for 4 weeks.

Within 3-4 weeks the animals were sacrificed and 4 mm punch biopsies were taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and/or transferred to cryotubes and snap-frozen in liquid nitrogen and stored at 5 -80EC.

Significant histological improvement marked by reduction of hyperkeratosis, acanthosis and lymphonuclear cellular infiltrates was observed in mice treated with the antisense oligonucleotides. Rete pegs, finger-like projections of the 10 epidermis into the dermis, were also measured. These are phenotypic markers for psoriasis which lengthen as the disease progresses. The shortening of these rete pegs are a good measure of anti-psoriatic activity. In mice treated with the active agent, the rete pegs changed from $238.56 \pm 98.3 \mu\text{m}$ to 15 $168.4 \pm 96.62 \mu\text{m}$ ($p < 0.05$), whereas in the control group the rete pegs before and after treatment were $279.93 \pm 40.56 \mu\text{m}$ and $294.65 \pm 45.64 \mu\text{m}$, respectively ($p > 0.1$). HLA-DR positive lymphocytic infiltrates and intraepidermal CD8 positive lymphocytes were significantly reduced in the transplanted 20 plaques treated with the antisense oligonucleotide cream. These results show that antisense oligonucleotides to B7 inhibit psoriasis-induced inflammation and have therapeutic efficacy in the treatment of psoriasis.